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Natural Resource Utilization for Sustainable Production and Food Security

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EDITOR'S NOTE

The Central Bicol State University has been one of the prime movers of regional development. It will continue to be an agent of societal transformation and development through the conduct of innovative and relevant research and extension activities that primarily aims to improve the Filipinos' way of life.

Inequality, poverty, and climate change are only three of the many complex problems faced in the world. That is why it is crucial for CBSUA to be part of the local policy process to foster evidence-based approaches in tackling these issues and form solutions to better address such problems. As a research university and in order to achieve its goals, the conduct of researches is sustained but not forgetting. respect for intellectual property, carefulness, honesty, and objectivity.

The AGRIKULTURA: CBSUA Research and Innovation Journal (ACRIJ) has published its 1st issue which featured researches on Philippine heirlooms such as pili, hot pepper (chili), mushroom, taro, gumamela, and stingless bees. Articles are research outputs of CBSUA's faculty-researchers and students. The 2nd issue of the ACRIJ will feature not only outputs of the university researchers but also papers from Cebu, Philippines, and Padang, Indonesia. With its theme "Natural Resource Utilization for Sustainable Production and Food Security", this issue features utilization of predators for the control of *Nilaparvata lugens,* damage assessment of miriid bugs on cacao, organic fertilization of tomato, responses of *India Echinacea* light spectrum, and carbon sources, and classification as well as identification of stingless bees in the Philippines. In addition, a touch of internationalization initiatives has featured a comparison of the ASIAN students' perception in generating innovative ideas. We are therefore grateful to the authors for entrusting their research outputs to us.

For this 2nd issue, we are indebted to the following Associate Editors who religiously reviewed the papers: Dr. Divina M. Amalin, Dr. Mark Ukwungwu, Dr. Nozomi Kawarazuka, Dr. Seneratne Ranamukhaarachchi, Dr. Rhodora R. Aldemita, Dr. Shaikh Tanveer Hossain, Dr. Srinivasan Ramasamy and Dr. Ravindra C. Joshi. Thank you so much.

As the Editor-in-Chief, I would like to thank the CBSUA President, Dr. Alberto N. Naperi for his full support of this endeavor. This issue would not be possible if not for the efficient and collective work of Dr. Ramona Isabel S. Ramirez (VPRI), Mr. Alvir Bausa, Prof. Julie Amara J. Mostoles-Bondilles, Catherine Almazar , and the rest of the Editorial Support staff.

MARIA DULCE J. MOSTOLES ,Ph.D.
Editor-in-Chief

FROM THE DESK OF THE UNIVERSITY VICE PRESIDENT FOR RESEARCH AND INNOVATION

Research publication is an important part of the research process. It is through it that scientific information is made publicly available and allowed the academic audience to access the value of the undertaking. Opportunities for career enhancement are also possible after publishing one's work.

The publication is required in many disciplines. Aside from promoting career improvements, it can also provide connections as well as an understanding of the field. Publishing likewise provides new opportunities, collaboration, and a higher level of professionalization.

Our AGRIKULTURA: CBSUA Research and Innovation Journal (ACRIJ) is an initiative that will provide an opportunity for our faculty-researchers and other professionals to enjoy the benefits. With the sincere intent of having a publication that will serve as a means of disseminating research results generated from the University research initiatives, ACRIJ is being maintained and processed for reputable indexing. In our 2nd issue, the quality of every paper published is maintained.

Along with it, the Office of the Vice President for Research and Innovation would like to extend its sincerest thanks and appreciation to the people who made this 2nd issue possible: to the CBSUA President, Dr. Alberto N. Naperi, for his consistent support; to all the reviewers who did not hesitate to share their expertise in polishing the papers; to our contributors; and to the editorial board, we will value your efforts forever. We believe that with your continued support ACRIJ will go a long way.

RAMONA ISABEL SALCEDO-RAMIREZ, Ph.D.
Vice President for Research and Innovation

CONTROLLING BROWN PLANT HOPPER (*Nilaparvata lugens* STÅL) BY JOINT PREDATORS (*Pardosa pseudoannulata* Boesenberg and Strand and *Verania lineata* Thunberg) UNDER COMPETITIVE CONDITIONS

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Abstract — *Pardosa pseudoannulata* and *Verania lineata* are two generalist predators commonly found in rice fields. Only a few references indicate their presence as joint predators resulting in positive interactions to brown planthopper without competition. The research aimed to determine the predation rate of joint predators in competitive conditions. The research was conducted in the laboratory using a Completely Randomized Design (CRD) with different combinations of joint predators. The density of *P. pseudoannulata* (P) and *V. lineata* (V) were 1, 3, 5 individuals per treatment (P1V1, P1V3, P1V5, P3V1, P3V3, P3V5, P5V1, P5V3, and P5V5). Each treatment had five replications. The results showed that competition and cannibalism factors indicate a negative interaction that affected the predation rate of joint predators. The ability of *P. pseudoannulata* to survive in competitive conditions was lower than *V. lineata*. The suitable and safe combination was using one (1) *P. pseudoannulata* and three (3) *V. lineata* (P1V3) with 89.6% predatory rate on *Nilaparvata lugens* Stål on the first day, and with the lowest death rate of two predators. The P1V3 combination also had an increase in bodyweight of *P. pseudoannulata* and a competition model that resulted in draw conditions. Therefore, before using some predators to control the BPH optimally, there is a need to minimize the impact of competition and cannibalism on them.

Keywords **— Brown planthopper, rice, lady beetle, wolf spider, prey consumption, cannibalism, competition.**

INTRODUCTION

Brown planthopper or BPH (*Nilaparvata lugens* Stål, Hemiptera: Delphacidae), is the primary pest of rice worldwide. Its attack causes losses and crop failures in many countries. The first attack of BPH in Indonesia that causes "hopperburn" occurred in 1980s, and there is an increase of attacks every year (Baehaki and Mejaya, 2014). In West Sumatra, one of provinces in Indonesia, the attack increases rapidly during 2015-2017, followed by attacks of grassy rice stunt and ragged stunt viruses (Food Crops and Horticultural Protection Agency of West Sumatera, 2019). Controlling BPH using natural enemies such as predator has not been the best choice for farmers because the results are not measurable, and the method does not guarantee population suppression of BPH, as is the case with synthetic pesticides.

The effectiveness of single predators, such as *Pardosa (syn= Lycosa pseudoannulata* (Araneae: Lycosidae) and *Verania* (syn= *Micraspis*) *lineata* (Coleoptera: Coccinellidae) has been reported widely (IRRI, 1982; Heong et al., 1990; Preap et al., 2001; Laba, 2001; Syahrawati et al., 2015). *P. pseudoannulata* is a generalist predator that does not have specific preferences for prey and tends to catch the closest abundant prey (Reissig et al., 1985; Riechert and Lawrence, 1997; Foelix, 2011). Furthermore, *P. pseudoannulata* is polyphagous predator, very generalist, and time generalist, i.e., this species consumes the prey without time limitations (Suana and Haryanto, 2013). On the contrary, *V. lineata* is a generalist predator, but it has specific prey preferences (Karindah, 2011). Yasuda and Kimura (2001) and Snyder et al. (2004) reported that spiders and lady beetles are top predators and intraguild predators.

Based on some experiments on single predation in the laboratory, *P. pseudoannulata* can consume 5-15 BPH per day (Shepard et al., 1987; Heong et al., 1990; Lubis, 2005; Syahrawati et al., 2015), while *V. lineata* can consume 1-11 BPH per day (Lubis, 2005; Karindah, 2011; Syahrawati et al., 2015). The data generally used to predict the success rate in controlling BPH, assuming that increasing species and number of predators will increase the suppression on pests in the field by sharing prey (Morin, 1999; Riechert, 1999; Sembel, 2010). Only a few studies have shown that joint predators can significantly suppress the BPH population. Therefore, research on this topic needs to be done.

Letourneau et al. (2009) stated that controlling pests using organisms with higher trophic levels can be beneficial if the natural enemy species are complementary, suitable, and there are no negative interactions. However, with of *P. pseudoannulata* as well and as *V. lineata* are generalist predators, the potential for competition in the field is very high (Snyder and Ives, 2001; Lucas, 2005), which could influence their predatory behavior and the consumption rate. Syahrawati et al. (2015) reported that the presence of joint predators like the *V. lineata* and *P. pseudoannulata* simultaneously causes a sublethal effect that harms both predators, but they can still suppress BPH populations more than 80% without intraguild predation (IGP). Lucas (2005) described three possibilities that occur in interspecific competition: Killing and preying on competitors, killing but not preying on competitors, or killing but causing sublethal effects.

Preap et al. (2001) reported that joint predation by *P. pseudoannulata* predation and *Araneus inustus* increase the pressure on the BPH population. The predation rate *of P. pseudoannulata* is higher

than *A. inustus* two times. The optimum ratio between predators and BPH is 1:11 to 1:20. Furthermore, the effective and suitable composition of joint predators between *P. pseudoannulata* and *V. lineata* is not yet known. Varshney and Ballal (2019) stated that it is needed to select species compositions based on positive interaction so that they can be used synergistically to enhance the efficacy of biological control. This research was conducted to determine joint predators' ability to suppress BPH and to find the suitable composition of them in competitive conditions.

MATERIALS AND METHODS

Predator Collection

P. pseudoannulata and *V. lineata* (Figure 1) were collected using small bottles directly from rice fields in Pauh District, Padang, West Sumatera in June 2019. The predators were transferred into a plastic cup separately to avoid cannibalism and fed three to five nymphs of *N. lugens* at the Laboratory of Insect Bioecology, Faculty of Agriculture, Universitas Andalas. Each time the prey was already consumed, an additional prey was added . The predators were starved for 24 hours before use after one week. There were 135 individuals of *P. pseudoannulata* and 135 individuals of *V. lineata* of a similar size provided for all treatments and replications.

Fig.1.The two predators used in the study; Left. *Pardosa pseudoannulata* (body length = 7.1 mm), Right. *Verania lineata* (body length = 4.7 cm).

N. lugens Rearing

N. lugens was reared in the laboratory at the beginning of October 2017 on IR 42 variety. The seeds were soaked for 24 hours and then air-dried for ± 1 hour and transferred to a culture jar (volume = 25 liters) containing water as high as 2 mm. The water height was maintained in that position, covering the whole seeds. Five to seven days after sowing, ten pairs of *N. lugens* adults were placed into a culture jar. Seven to ten days later, the first nymph instar emerged. It was needed 50 individuals for each treatment. The total number of nymphs (2^{nd} to 3^{rd} instars) needed in this study was 2, 250 individuals (Figure 2).

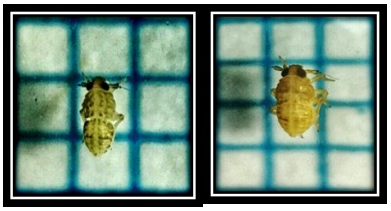

Fig. 2. The nymphs of *Nilaparvata lugens* used in the study; Left. 2^{nd} instar (body length = 1.2 mm), Right, 3^{rd} instar (1.8 mm).

Treatments

The study was conducted at the Laboratory of Insect Bioecology, Faculty of Agriculture, Universitas Andalas, from June-August 2019. The average minimum and maximum daily temperatures were 26.7°C and 29.2°C, respectively, while the average minimum and maximum daily relative humidity were 70.6% and 80.9%, respectively. The study used a Completely Randomized Design (CRD) with treatments in the form of different compositions of the two predators (P = *P. pseudoannulata*, V= *V. lineata*), such as 1, 3, and 5 individuals of each predator (P1V1, P1V3, P1V5, P3V1, P3V3, P3V5, P5V1, P5V3, P5V5). Each treatment was done in five replications.

Two plastic cups with a volume of 360 ml were provided for each replication. One cup was perforated at the bottom using a hot nail with a diameter of approximately 2 mm. Three rice seedlings aged seven days were placed into the cup through a hole, while the roots were positioned outside the cup. The second cup was filled with water with a height of 2 mm and placed overlapping with the first cup to be used as root growth media. Then, the nymphs of *N. lugens* were placed into the cup according to the treatment. Joint predators that had been starved for 1 X 24 hours and weighed before use in the treatment using analytical scales with a precision of 4 decimals.

Data Gathered

Predation. The number of *N. lugens* consumed by joint predators was determined by counting the number of *N. lugens* consumed for 3x24 hours. Then, the percentage of predation was computed using the following formula:

$$P = \frac{n}{N} \times 100$$

where, P is Predation, n is Number of *N. lugens* consumed, and N is Number of *N. lugens* provided.

Joint Predator Mortality. The mortality of the two predators was counted for 3x24 hours and then measured using the following formula:

$$M = \frac{nm}{Np} x\ 100$$

where M is Mortality (%), nm is the Number of predators dead, and Np is the Number of predators provided.

Bodyweight (g). The body weight of each predator was measured by weighing it using analytical scales with a precision of four decimals. The bodyweight gain was obtained by measuring the difference in the bodyweight in the 1st to 3rd day compared to the bodyweight before treatment.

Competition Model. Competition between two predators was observed in a plastic cup within 1 x 24 hours, and then a quantitative assessment was given according to Table 1.

Table 1. The criteria to determine competition model between two predators (*Pardosa pseudoannulata* and *Verania lineata*) in the laboratory.

Value	Criteria	Meaning
0	Lost	One predator is dead, while all competitors are still alive or the mortality percentage of one predator are higher than that of the competitor.
1	Draw	All predators are alive or all predators are dead.
2	Win	There are deaths of both predators, but the mortality of one predator is less than that of the competitor.
3	Very Win	One predator is still alive but the others are all dead.

Data Analysis

The data about predation rate and predator mortality were statistically analyzed with analysis of variance (ANOVA), and the significant difference was tested under CRD design at probability level 0.05% by using statistic 8 software. The data are displayed in a table with the standard error. Furthermore, the bodyweight gain data and competition model are displayed in the form of graphics.

RESULTS AND DISCUSSION

Predation Rate (Individuals)

The predation rate of joint predators at different densities ranged from 43.2-49.4 individuals of BPH (86.4-98.8 %) in one-day observation. The highest predation rate occurred for P5V5, but it was assumed that it was not safe because of the cannibalism between the predator *P. pseudoannulata*, which did not occur for *V. lineata*, having the predation rate not much higher than other combinations. The predation of joint predators on BPH increased with the constant number of one individual of *P. pseudoannulata* , but the number of *V.lineata* increased. Increasing the *P.pseudoannulata* to three individuals resulted to a decrease in the predation rate, even as the number of *V.lineata* increased, and fluctuating when the number of *P.pseudoannulata* was increased to five individuals (Table 2).

In the P1V1, P3V1, and P1V3 combinations, joint predators tended to consume all prey until the third-day observation, but predators in the other combinations consumed their prey on the second day (Figure 3).

Bodyweight Gain of Joint Predators (g)

The bodyweight gain of predators after the third observation day was higher in *P. pseudoannulata* than *V. lineata.* The bodyweight of *P.pseudoannulata* fluctuated, except for P1V3, P1V5, and P3V3 combinations, and the increase in bodyweight of *P. pseudoannulata* in P3V3 was higher than *P.pseudoannulata* in P1V3 and P1V5. Meanwhile, the bodyweight of *V.lineata* decreased, except for P3V3 and P5V3 combinations, but the increase in bodyweight of *V. lineata* in P3V3 was higher than *V. lineata* in P5V3. The P1V3 combination was classified as a suitable and safe for joint predators because the bodyweight gain was only reached by consuming BPH, not from cannibalism or intraguild predation (Figure 4).

Table 2. The predation rate of joint predators (*Pardosa pseudoannualata* and *Verania lineata*) on *Nilaparvata lugens* under competitive conditions (one-day observation).

Joint Predator Group	Predation (Individuals) ± SE	Predation (%) ± SE
P1V1	43.2 ± 2.7	86.4 ± 4.9
P1V3	44.8 ± 2.9	89.6 ± 5.9
P1V5	45.8 ± 1.9	91.6 ± 3.8
P3V1	47.2 ± 1.3	94.4 ± 2.7
P3V3	45.6 ± 1.8	91.2 ± 3.6
P3V5	45.2 ± 0.6	90.4 ± 1.2
P5V1	48.0 ± 0.8	96.5 ± 1.7
P5V3	47.6 ± 1.1	95.2 ± 2.1
P5V5	49.4 ± 0.4	98.8 ± 0.8

Note: SE = standard error

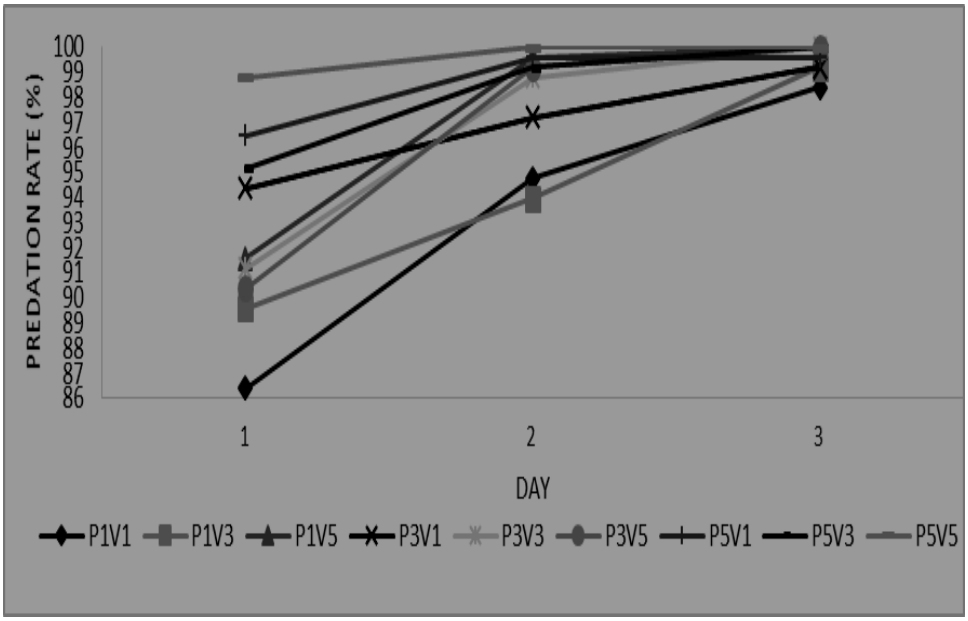

Fig. 3. Accumulated predation percentages of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) on *Nilaparvata lugens* under competitive conditions during three days.

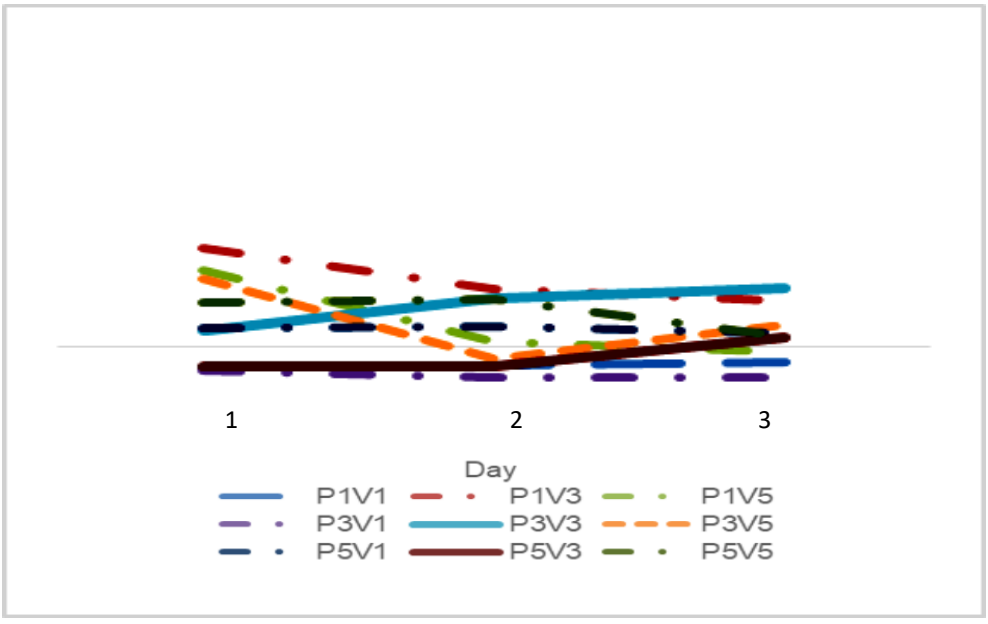

Fig. 4. The development of joint predator weight (g). (Left: *Pardosa pseudoannulata*, Right: *Verania lineata* after consuming *Nilaparvata lugens* during three days observation.

Joint Predator Mortality

Unfortunately, the attempt to suppress the BPH population by using joint predators at different densities resulted in harmful and even lethal competition (competitors killed but did not prey on each other). The deaths of both P. pseudoannulata and V.lineata fluctuated among treatments. The P1V3 composition of joint predators was classified as safe, with mortalities of 0 and 0.2 individuals of the two predators (Table 3).

Cannibalism and competition increased the mortality of joint predators during three days observation, with the highest impact found for *P. pseudoannulata* having High mortality than *V. lineata*. Increase in mortality to 73.33% of *P. pseudoannulata* while *V. lineata* mortality reached 46.67% on the third -day observation. There was no death on *P. pseudoannulata* in P1V1, and there was no death on *V. lineata* in P5V1, but the highest mortality of both predators occurred in the highest combination, that is, in P5V5. In this condition, cannibalism and competition occurred between individuals of *P. pseudoannulata,* whereas and weak competition occurred between *V. lineata* individuals (Figure 5).

Competition Model

The survival ability of *P. pseudoannulata* was lower than *V. lineata*, with a high mortality rate. V. li*neata* obtained a winning scenario in almost all treatments. As observed during the three-day observation period, *P. pseudoannulata* won the competition when the density remained at one individual, while *V. lineata* continued to increase. When the density of both predators was added, *V. lineata* won the competition. The draw competition was seen in the density of P1V1 and P1V3 combinations. This was because unlike *P. pseudoannulata*, the predator *V. lineata* did not exhibit cannibalistic behavior. The presence of *P. pseudoannulata* did not serve as threat to *V.lineata* unless the former made a netting to move to another site, not to trap the prey or the competitor.

Table 3. Mortality of joint predators (*Pardosa pseudoannulata* and Verania *lineata*) on *Nilaparvata lugens* under competitive conditions (one-day observation).

Joint Predator Group	*P. pseudoannulata* Mortality (Individuals) ± SE	*V. lineata* Mortality (Individuals) ± SE
P1V1	0.2 ± 0.2	0.2 ± 0.2
P1V3	0 ± 0	0.2 ± 0.2
P1V5	0 ± 0	0.4 ± 0.2
P3V1	1.0 ± 0.4	0.6 ± 0.2
P3V3	0.6 ± 0.3	0.2 ± 0.2
P3V5	1.2 ± 0.5	0.4 ± 0.2
P5V1	1.8 ± 0.8	0.2 ± 0.2
P5V3	1.2 ± 0.5	0.2 ± 0.2
P5V5	1.8 ± 0.4	1.0 ± 0.3

Note: SE = standard error

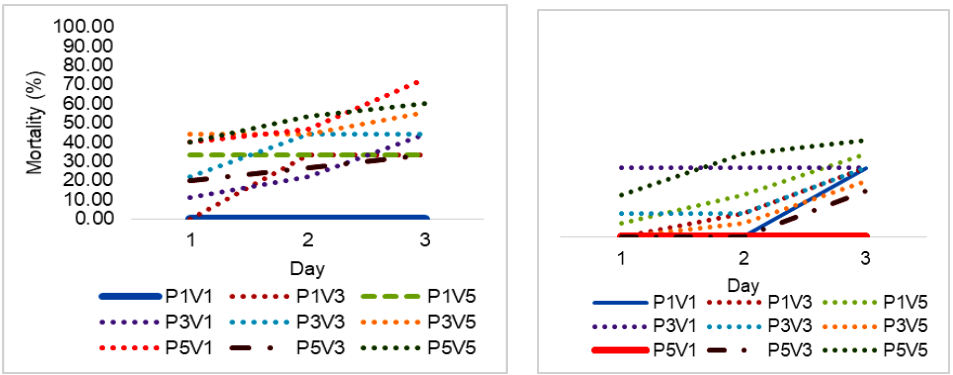

Fig. 5. Joint predator mortality in suppressing *Nilaparvata lugens* under competitive conditions (Left: *Pardosa pseudoannulata* mortality; Right: V*erania lineata* mortality) during three days observation.

The netting often trapped *V. lineata* in flight. However, when *V. lineata* was trapped, *P. pseudoannulata* did not try to prey on it (Figure 6). Both *P. pseudoannulata* and *V. lineata* are two species of generalist predators from different taxonomic classes, and both are often found together in rice fields (Syahrawati et al., 2014). Previous research revealed that there is a sublethal interaction between the two predators, but they have a potency to be joint predators due to their behavior in finding prey (Syahrawati et al., 2015). From this research, a novel information was obtained that competition and cannibalism indicate a negative interaction between the two predators which in turn affected the predation rate although the differences were not significant.

The predation rate of joint predators increased with only one individual of *P. pseudoannulata*, even though the *V. lineata* increased in number. Cannibalism did not occur between individuals in *V. lineata* but only occurred between individuals of *P. pseudoannulata*, while competition occurred between inter- and intra-predators. The P1V3 combination was classified as suitable and safe because the two predators in this treatment were able to consume 89.6% of BPH on the first day and tended to consume all prey until the third days observation. The P1V3 combination also had an increase in the bodyweight of *P. pseudoannulata*, the lowest joint predator mortality, and a competition model that resulted in the draw condition. Approximately 62% of interactions among arthropods in the world are in the form of competition (Kaplan and Denno, 2007). Competition for nutrition does not only occur between predator and predator but also between predator and parasitoid as reported by Hussaini and Askar (2019) and Varshney and Ballal (2019). The competition can decrease or increase the predation rate or disturb the existence of competitors (Foelix, 2011; Syahrawati et al., 2015).

On the other hand, it can trigger an increase in the herbivore populations due to reduced pressure from natural enemies that prey on one

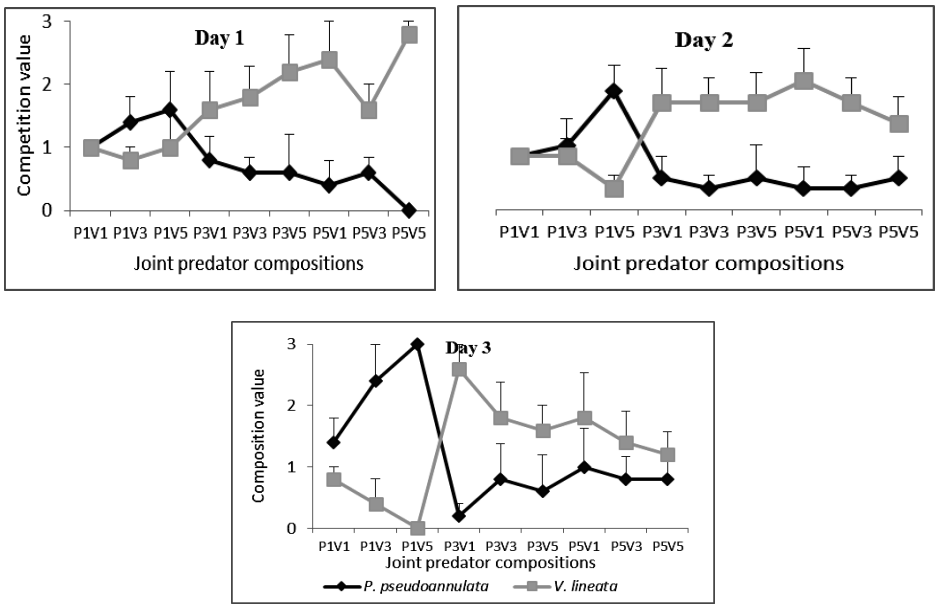

Fig. 6. Competition model of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) to suppress *Nilaparvata lugens* population at different densities (3 days observation).

another (Denno et al., 2002). Very strong competition can reduce the predation rate, whereas a weak competition can increase the predation rate and pressure on prey (Menge and Sutherland, 1976; MacArthur, 1984).

Furthermore, the cannibalism incidence is affected by the availability of food, starvation, densities, size, and foraging behavior (Wagner and Wise, 1997; Samu et al., 1999; Rickers and Scheu, 2005; Gonzalez, 2012). Cannibalism generally occurs when large or heavy spiders cannibalize smaller or lighter spiders (Gonzalez, 2012), due to the fear of retaliation (Hvam et al., 2005), when females cannibalize males to increase the amount of offspring (Wu et al., 2013) or when females cannibalize young females (Torres-Contreras et al., 2015). Cannibalism can occur when food is rare (Polis, 1981), food is abundant (Wagner et al., 1999), or there is poor-quality prey (Snyder et al., 2000). Then, cannibalism also occurs among Coccinellidae, especially on eggs and larvae (Khan et al., 2003; Burgio et al., 2005; Pervez et al., 2006; Bayoumy and Michaud, 2015a; Bayoumy and Michaud, 2015b), but not between adults (Syahrawati et al., 2015).

CONCLUSIONS

This study demonstrated that competition and cannibalism factors indicate a negative interaction that affected joint predators' predation rate. The ability of *P. pseudoannulata* to survive in a competitive condition was lower than *V. lineata*.

The P1V3 composition (one *P. pseudoannulata* and three *V. lineata)*

was suitable and safe because the predators preyed on the BPH up to 89.6% on the first day and reached the lowest mortality of both predators.

The P1V3 (five *P. pseudoannuata* and five *V. lineata*) composition also increased the *P. pseudoannulata* bodyweight and a competition model that resulted in the draw condition. Therefore, before using some predators to control the BPH optimally, there is a need to ensure the impact of competition and cannibalism.

.ACKNOWLEDGMENTS

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MIRID BUG (*Helopeltis bakeri* P.) DAMAGE ON CACAO (*Theobroma cacao*) IN THE MAINLAND PROVINCES OF BICOL, PHILIPPINES

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Abstract — *Helopeltis bakeri* P., cacao mirid bug is one of the upcoming pests in the Philippines which results in the reduction of quality cocoa beans intended for processing. Sustainable pest management is essential for the effective control of this pest. Monitoring of its infestation in the plantations and the extent of pod damage is important information for the success of its control. Thus, the infestation of *H. bakeri* was monitored in selected municipalities in the mainland of the Bicol region, for a period of three months. Percentage-infested trees and damage ratings on cacao pods were recorded. Mirid bug-infested tress in cacao plantation was obtained at Gubat, Sorsogon with 23.85%, Guinobatan, Albay with 35%, Sipocot, Camarines Sur with 34.48%, and Labo, Camarines Norte with 0%. Pod damage on cacao along the three monthly monitoring periods increased in Sorsogon from 70.83% to 87.50%, decreased in Albay with 93.75% to 48.75%, and decreased in Camarines Sur from 87.92% to 85.42%. Zero pod damage in Camarines Norte for the period of 3 months. Continuous monitoring of the cacao mirid bug infestation and cacao pod borer occurrence is recommended as the basis for timely control.

Keywords — **Cacao, Bicol region, mirid bug, pod damage**

INTRODUCTION

Cacao (*Theobroma cacao* L.) today has been raised as a high-value commercial crop with total volume production of 37.98 metric tons throughout the Bicol Region in 2016. A reported average cacao production of 600 kilos per hectare in the Bicol region was while 909 kilos per hectare for the Philippines (DA RFO-5). Recently, production and global market demands for cacao beans are still increasing. However, in 2015, the International Cocoa Organization (ICO, 2015) estimated a 30 to 40 percent loss in cacao production due to insect pests and diseases. These losses pose an impact throughout the cacao supply chain, thereby having the most direct effect on family income.

Gavara (1990) recorded the presence of pests in the Philippines. There are four major pests namely: cacao pod borer, cacao mirid bug, black pod rot, and vascular streak dieback. In Davao 60-80% infestation from cacao pod borer was recorded. The cacao mirid bug was recorded in Quezon, Davao City, Zamboanga del Norte, and Aklan but no concrete data has been recorded on its possible infestation, however, it was claimed that infestation can be as high as 90% is uncontrolled. Recently, it has been identified as a major pest of cacao in Luzon. This mirid species prefers to feed and oviposit on cacao pods causing characteristic feeding lesions through which pathogens such as Phytophthora spp. can easily enter (Amalin, 2017).

There are four (4) species of *Helopeltis* have been reported in the Philippines, among which *H. bakeri* Poppius has most recently been identified as a major insect pest of cacao in Luzon. This particular mirid species prefers to feed and oviposit on cacao pods, causing characteristic feeding lesions damage (Amalin, 2017). The cacao mirid bug completes its life cycle in about 4-6 weeks. The females lay eggs in the stems or pods of the cacao tree. The nymph emerges after 13-18 days and is only about 3 mm long. After feeding for about four days, it is fully grown with its skin splits to the next nymph stage. The nymph passes through 5 instars over a period of 4 weeks before developing into winged adults. The adult female begins to lay eggs 4-11 days after mating and they can lay a total of about 200 eggs over a period of 30 days that are embedded in the bark of stems or inside the pod husk and may live a few weeks afterward, while the adult males live for two or three weeks (ICCO, 2015).

The damage inflicted on the growing vegetative parts causes the branches to dry out. Losses due to mirids are difficult to estimate, but can reach 30–40% of potential production in other countries (Adu-Acheampong et al., 2014 ; Awudzi et al., 2016).

Mirid adults and nymphs suck the sap from cocoa pods producing 3 mm diameter spots, which are brown at first then turn black. Young pods (cherelles), which are less than 10 cm long are destroyed, more mature pods are deformed and beans are smaller than those in healthy pods. Mirid destruction, if left unattended for three years, can reduce yields by as much as 75%. Cacao mirids pierce the surface of cacao stems, branches, and pods, killing the penetrated host cells and producing unsightly necrotic lesions. Mirids feeding on shoots often results in the death of terminal branches and leaves that vascular causing dieback (ICCO, 2015). Heavy infestations of mirid bugs can result in pod malformation and premature drop. Feeding on young pods sometimes results in pod wilting, since the feeding puncture provides an avenue for the second invasion of microorganisms. Thus, mirid bug can cause a significant loss in yield.

Today, various control measures such as insecticide applications, sanitation, pruning, and the use of biological control agents are being practiced but without complete control of insect pests and pathogens under the farmers' level. Additional productivity costs through unnecessary spraying without knowing the exact information on the pest status were encountered.

This study is taking into consideration some of the problems encountered by cacao farmers/growers on the *H. bakeri* as a major insect pest of cacao. The objective of this study is to determine the extent of pod damage of *H. bakeri* in cacao plantations in the four mainland provinces of the Bicol region. The study was delimited to the determination of the extent of pod damage by *H. bakeri* on cacao in plantations at Sorsogon, Albay, Camarines Sur, and Camarines Norte from October to December 2017.

MATERIALS AND METHODS

Site Description

The field assessment of *H. bakeri* was conducted in municipalities with cacao plantations in the four mainland provinces of the Bicol region. The municipalities identified were the following: Gubat in Sorsogon, Guinobatan in Albay, Sipocot in Camarines Sur, and Labo in Camarines Norte.

The municipality of Gubat is the third-largest town in the province of Sorsogon. The total area planted was 0.5 hectares of pod bearing cacao intercropped with coconut trees. The area is located at Barangay Ariman, Gubat, Sorsogon.

Guinobatan is a municipality in the province of Albay and is located at 13°11′N 123°36′E. According to the Philippine Statistics Authority, the municipality has a land area of 244.43 square kilometers (94.37 sqm) constituting 9.49% of the 2,575.77-square-kilometer (994.51 sqm) total area of Albay. The territory of Guinobatan is bordered by several municipalities: Camalig on the east, Jovellar on the south, Pio Duran on the south-west, Ligao on the Northwest. On the northeast, the town shares with Malilipot, Sto. Domingo, Daraga,Tabaco and Legazpi. Area planted to cacao is more or less 5.0 hectares with pod bearing stage intercropped with coconut trees. The area is located at Barangay Quibongbongan, Guinobatan, Albay.

Sipocot is a municipality in the province of Camarines Sur; its geographical location is 13° 46' 13" North and 122° 58' 52" East. The total area planted is 1.0 hectare with pod bearing stage and intercropped with coconut trees. The cacao area is located at Barangay Impig, Sipocot, Camarines Sur.

The municipality of Labo is geographically located relatively at the center of the province and capital town of the province of Camarines Norte. It is approximately 335 kilometers south of Manila and 15 kilometers away from Daet. It is situated at the coordinates between 14°01'06" and 14°11'00" North latitudes and 122°21'00" and 122°52'20" East longitudes. On the North, it is bounded by the municipalities of Paracale, Jose Panganiban, and Capalonga, on the South, by the province of Quezon, adjoining province of Camarines Sur, on the East by the municipalities of Vinzons and San Vicente, and on the West by the municipality of Sta. Elena. The municipality is generally rugged, rolling hills and mountainous terrain with relatively small rollings and flat terrain. The cacao area is located at Barangay Mapaod, Labo, Camarines Norte.

The Extent of Pod Damage of *H. bakeri*

The extent of pod damage by *H. bakeri* on cacao was conducted in cacao growing plantations in each municipality. The basis for the selection of cacao plantations in the municipality is more or less 1.0 hectare or 500 cacao in the pod-bearing stage. For every farm, only three cacao trees were. evaluated obtaining 10 pod samples per tree. Monitoring and evaluation were carried out for a period of three months. In order to assess the feeding of *H. bakeri*, percentage pod damage was done on the exposed side of the cacao pod, which was divided into four quadrants as testing chambers (Figure 1).

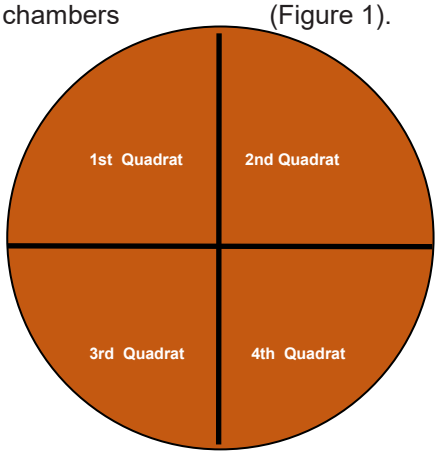


Fig. 1. Quadrats from where damage rating was done.

The damage rating was made using the was based on the number of quadrants with insect feeding, as follows:

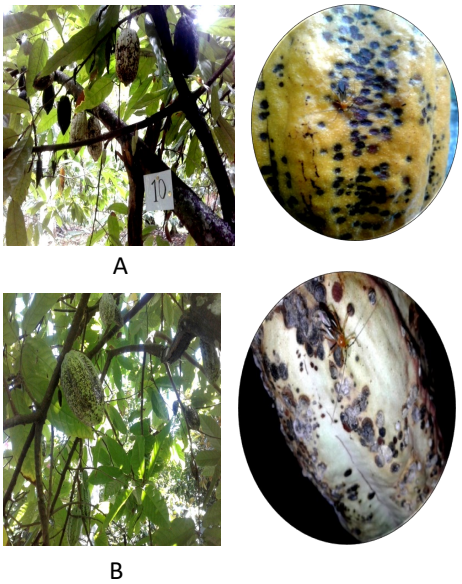
0 = no damage (no lesions observed)
1 = 1 quadrant with lesions (1 – 25% damage)
2 = 2 quadrants with lesions (26 – 50% damage)
3 = 3 quadrants with lesions (51 – 75% damage)
4 = 4 quadrants with lesions (76 – 100% damage)

RESULTS AND DISCUSSION

Survey of Mirid Bug-Damaged Cacao Trees

At the Municipality of Gubat, the cacao trees in the area were damaged by the *H. bakeri.* Out of 520 cacao trees, only 124 trees were damaged by H. bakeri with percentage damage of 23.85%. This damage occurred because the farmer did not practice pruning or any control measures. Farmer in the area claimed that they were not aware that cacao pods were infested by *H. bakeri* and they were unable to identify the insect pest as well. Moreover, only *H. bakeri* caused severe damage to cacao and also disease such as pod rot causing 20% damage. Shown in Figure 2A are the pods infested by mirid bugs in the area.

For the Municipality of Guinobatan, Albay, the cacao trees in the area were likewise infested by the *H. bakeri.* Out of 1,000 cacao trees, only 350 cacao trees were damaged by *H. bakeri* at a computed infestation of 35.0%. (Figure 2B). This damage occurred because the farmer did not practice pruning or any control measures. Farmer in the area said that they did not know the control measures of insect pest of cacao and they were unable to identify the insect pest as well. It was observed in the area that even cherelles (young pods) were already infested by mirids which caused black circular lesions to the cacao pod. Aside from *H. bakeri* damage, cacao pods were also infected by pod rot disease at any stage of development and the initial symptoms are mummified on any part of the pod. Internal tissues, including the beans, are colonized and shrivel to form a mummified pod and disease incidence varies with cultivar, pod age, and rainfall. Usually, the greatest production is when rainfall is high (ICCO, 2015).



A

B

Fig. 2. Cacao pods damaged by *H. bakeri* in cacao farms at Gubat, Sorsogon (A) and Guinobatan, Albay (B).

At Sipocot, Camarines Sur, the cacao trees in the area were infested by *H. bakeri.* Out of 580 cacao trees, only 200 cacao trees were damaged pegged at an infestation of 34.48%. Shown in Figure 3 are the damaged pods with lesions covering the entire pods. This damage occurred because the farmer did not practice pruning or any control measures. Farmer in the area said that they did not know the control measures of insect pest of cacao and they were unable to identify the insect pest as well. Other despite observed in the area that even cherelle or young pods were already infested by *H. bakeri* which caused black circular lesions to the cacao pod. The damage done by mirids is thought to be mechanical destruction of the cells around the stylets or feeding tubes, the loss of cell sap, as well as damage caused by chemicals in the saliva injected as the mirids feed.

It has been shown that the saliva of cacao mirid bug can remove cell contents without mechanically damaging the cells and this can happen up to 3.5 mm from the stylet. It is presumed that this occurs by osmosis. Aside from the *H. bakeri* damage, the cacao pods were also infected by pod rot disease.

Fig. 3. Cacao pods damaged by *H. bakeri* in cacao farms at Sipocot, Camarines Sur.

At the Municipality of Labo, Camarines Norte, the cacao trees in the area were found to have no damage by *H. bakeri.* Out of 650 cacao trees, there was no indication of pod damage (0%). Shown in Figure 4 are the cacao trees and pods without indication of the presence of mirid bugs. It was obtained that *H. bakeri* is not present in the area instead a few pods were infected with pod rot.

Cacao was intercropped with banana and the farmer said they practice the proper pruning measures and fertilizer management on the cacao tree that learned from training.

Fig. 4. Healthy cacao pod tree in cacao farm at Labo, Camarines Norte.

Extent of Pod Damage by Mirid Bug

Mirid bugs cause an adverse effect on the physical appearance of the cacao pods. During the early stage of its infestation, the cherelles fall off while more mature pods exhibit a variety of symptoms. There are however cases wherein inner parts of the cacao pods are spared from damage thus cacao beans are still marketable.

For Gubat, Sorsogon, the extent of pod damage is shown in Figure 5 and samples with lesions of *H. bakeri* damage in Figure 6. The percentage of pod damage for the 1st monthly sampling (October) was 70.83%. There was an increase in the pod damage for the succeeding months with 83.75% for November and 87.50% for December. This increase in pod damage from 1 to 3 months occurred because the farmer never practiced control against the pests and regular monitoring was not done.

The cacao trees were fully shaded by a canopy that caused a higher incidence of *H. bakeri.* Overshading is the favorable breeding site for mirids resulting in higher populations in the cacao area (personal observation). In plantations, mirids gather in open canopy zones, known as mirid pockets. Population densities, although fairly low overall, reach maximum during the pod growth period.

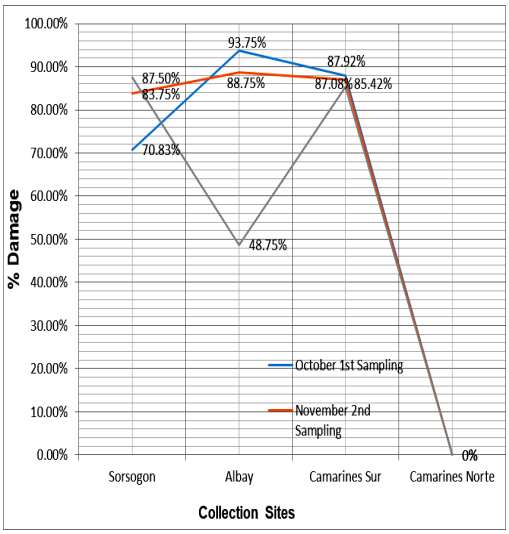


Fig. 5. Extent of pod damaged by *H. bakeri* in cacao farms in the four mainland provinces in the Bicol Region.

Fig. 6. Pod damaged by *H. bakeri* in the province of Sorsogon.

For Albay, the extent of pod damage by *H. bakeri* is shown in Figure 7. The percentage of pod damage for October was high at 93.75% which decreased in November (88.75%) and further decreased in December (48.75%) (Figure 5). The high pod damage occurred only in the 1st and 2nd samplings since the farmer did not apply control against the pest. Likewise, the fully shaded condition in the plantation with low hanging branches was favorable for the increase in population, thus, inflicting more damage. Though the farmer conducted regular monitoring of the pods in the cacao area, there was still high damage on to pods recorded. Shade and canopy management should be designed to attain a balance between mirid bug control, flowering, and black pod management. Another host should not be used as shade trees on cacao farms (ICCO, 2015).

1st 2nd 3rd

Sampling Periods

Fig. 7. Pod damaged by *H. bakeri* in the province of Albay.

Mirid bugs usually occur on trees visible to sunlight since such trees tend to bear more fresh shoots and pods. Though the insect is attracted to trees exposed to sunlight, after tracing their source of food they inhabit shady areas on trees. Some native plants grown with cacao have been identified as alternative hosts for some species of mirid bug. In the 3rd sampling month, there was a sudden decrease in the percentage pod damage. During the first two sampling months, the researcher had the chance to discuss with the farmer some pest management strategies against the mirid bugs. One of the recommendations is corrective pruning measures to reduce the *H. bakeri* feeding activities to regulate the populations of the *H. bakeri* in the cacao pods. The farmer stated that pruning is a very effective method to control *H. bakeri* including the reduction in disease infestation (personal interview). It was reported in DropData (2014) that when pruning is done too severely, this will result to inflicting stress the cacao trees and cause the growth of new chupons, which increases the mirid feeding.

For Camarines Sur, the extent of pod damage by the mirid bug is shown in Figure 8. The percentage extent of pod damage in October was 87.92%, with a slight decrease in November (87.08%) and December (85.42%). The high percentage of pod damage for the three sampling months could be attributed to the pruning practices employed by the farmer. Improper pruning was done wherein the branches are thinned but the infected pod was left on the tree. This resulted in an increase in the incidence of pod rot.

1st 2nd 3rd

Sampling Periods

Fig. 8. Pods damaged by *H. bakeri* in the province of Camarines Sur.

The continuous feeding *of H. bakeri* on the pod lesions further caused the spread of the causal organism of pod rot. Thus, there was increased pod damage not only due to the mirid bugs but also due to pod rot.

There was no pod damage by *H. bakeri* in the cacao plantation at Labo, Camarines Norte for the entire duration of the study (October to December 2017) (Figure 9). The zero damage could be attributed to the absence of natural populations of *H. bakeri* since the plantation was just established. For a period of 4 to 5 years, there has no record of the incidence of the *H. bakeri*. It was also observed that the farmer practiced pruning and keen monitoring of pests.

In almost all the sites, the damage on pods by mired bugs appeared as dark, circular lesions usually hardening as scars on the husk. During heavy infestations, it results in pod malformation and premature drop. When young fruits are infested, pod wilts resulting from feeding on very young fruits. It was also observed that mirid bugs feed on cacao shoots occurs particularly on the midribs of leaves and on the stems. Thus, severe infestations result to shoot die-back (Amalin, 2017).

1st 2nd 3rd

Sampling Periods

Fig. 9. No pod damage by *H. bakeri* in the province of Camarines Norte.

The biology and control of this pest were carried out. Likewise, the infestation of this mirid bug in Camarines Sur has been done and among the farms surveyed by Ablitea (2017), cacao at Lupi area was entirely affected by mirid bugs with percent damage of 68% followed by Caroyroyan (Pili) (62.5%), San Juan (Del Gallego) (50.5%) and Carolina (Naga City) (50%). The lowest damage was found in Nabua with 42.8%. The farmers perceived that a symptom of mirid bug damage is not due to biotic but environmental factors. Farmers control the pests by using insecticides such as cyhalothrin and methomyl. Infected fruit is removed and piled in compost pits. Other cacao farmers just leave infected pods under the tree and wait for it to rot.

CONCLUSION

Infestation of *H. bakeri* , a major insect pest of cacao was observed in selected municipalities in the mainland of the Bicol region. Cacao plantation in Gubat, Sorsogon had a recorded mirid bug infestation of its trees at 23.85% while 35% of trees in a plantation at Guinobatan, Albay. For the plantation at Sipocot, Camarines Sur , 34.48% of the trees were infested while 0% for the cacao plantation at Camarines Norte. Monthly data obtained for the pod damage in the different plantations covering the months of October to December (2017) showed different trends. In Sorsogon, it increased from 70.83% to 87.50%, decreasing for Albay with 93.75% to 48.75%, and likewise decreasing for Camarines Sur from 87.92% to 85.42%. No infested pods were found in Camarines Norte for the period of 3 months.

RECOMMENDATIONS

Continuous monitoring of the occurrence and infestation by the cacao mirid bug must be done in the Bicol region and sustainable control strategies must be employed.

Considering that numerous plantations are being established in the entire region, surveillance of these pests particularly the Cacao pod borer as well as the mirid bugs should be done. Training of cacao growers must be intensified granting that this study has identified the lack of knowledge on the biology, behavior, and control of the different pests of cacao. With these, there is an assurance of sustainable production of cacao in the region.

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YIELD AND QUALITY OF TOMATO FROM ORGANIC FERTILIZATION SYSTEMS IN THE PHILIPPINES

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Abstract — The effects of vermicompost and different organic concoctions on the yield and quality of tomatoes, determining the quality of tomato fruits in terms of ascorbic acid and beta-carotene contents as influenced by different organic fertilization systems was evaluated at Escuala, Casiguran, Sorsogon, Philippines (July- November 2017). The Randomized Complete Block Design (RCBD) was used with 15 treatments replicated thrice. Treatment was as follows: T1 - Farmer's practice (control); and T2 to T15 vermicompost (VC) as basal fertilizer. Results for the yield include the number of fruits, the weight of fruits/ plant, number of marketable fruits/plant, the weight of marketable fruits/plant, and number of non-marketable fruits/plant. Analysis of ascorbic acid and beta-carotene determined the quality of fruits. For each treatment, 100g samples of marketable tomato fruits at the 1st harvest were brought to the College of Agriculture, Food Science Cluster, University of the Philippines for ascorbic acid and beta-carotene content analyses. T1-Farmer's practice (Synthetic fertilizer) was significantly the same as T2-Vermicompost (Organic basal fertilizer) on different parameters of fruit yield. Among the different treatments, T14 (VC+IMO+FAA+CalPhos) yielded the highest number and weight of fruits per plant, number and weight of marketable fruits per plant, and fruits tons/hectare. T14 was similar with T13 (VC+IMO+FAA+FFJ) and T15 (VC+IMO+FFJ+CalPhos) significantly similar with T1 on the numbers of fruits per plant; and significantly different in almost all the other factors in the yield of tomato fruits. Organic fertilizer at T15 had the highest beta-carotene content in tomato fruits (47.80 mg/100g), followed by T2 (24.19 mg/100g), and T14 (19.56 mg/100g).

Keywords **— Ascorbic acid, beta-carotene, fruit yield, organic fertilization, tomato**

INTRODUCTION

Tomato (*Solanum lycopersicum* L.; *Lycopersicon esculentum* Miller*)* locally known as "kamatis" in the Philippines, is an edible, red berry-type fruit of the Nightshade family. It is the second most important vegetable crop next to potato (http://www.growtomatoes.com/tomato-world-production-statistics). In 2020, the top ten countries that processed tomatoes more than 83% of the quantities processed worldwide are California, China, Italy, Spain, Turkey, Iran, Portugal, Brazil, and Algeria (first time), and Tunisia (Branthome, 2020).

In the Philippines, the production of tomatoes for the period of January to March of 2019 reached 95.30 thousand metric tons, up by 3.9 percent from its 2018 level of 91.69 thousand metric tons. Ilocos Region, the major producer of tomatoes had 42.58 thousand metric tons or 44.7 percent of the total production this quarter. The other major producing regions were Central Luzon with 22.8 percent and CALABARZON, 8.9 percent (Bureau of Statistics Authority, 2019).

Tomato is a low-calorie vegetable equivalent to 18 calories per 100 g and it is an excellent source of antioxidants, dietary fiber, minerals, and vitamins. It is a good source of antioxidant vitamin C (providing 21 % of recommended daily levels per 100 g). Consumption of foods rich in Vitamin C helps the body develop resistance against infections agents and scavengers of harmful free radicals. It is also very rich in potassium (100 grams contain 237 mg of potassium and 5 mg of sodium). Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure. It also carries an average level of B-complex vitamins such as folates, thiamin, niacin, riboflavin, and some essential minerals like iron, calcium, manganese, and other trace elements (Rudrappa, 2017). Worldwide, there are 51 million hectares of certified organic agriculture land and 39 million hectares of wild culture land. In the last two decades, organic agriculture has been growing at 11.9% per annum, thereby doubling the size of the sector every six years (Paul, 2017). In the Philippines, organic agriculture has progressed tremendously as seen in the government's proactive initiatives and policies; research activities of state universities, and inclusion of organic agriculture subjects in the curriculum of colleges (Alleje and de Villa, 2014).

Organic farming lessens the use of synthetic fertilizers and restores the fertility of the soil for sustainable agriculture. Microorganisms are found to be useful and potent in eliminating problems associated with the use of chemical fertilizers and pesticides. Effective Microorganism (EM) technology helps farmers develop economically viable farming systems, and environmentally and socially acceptable. It contains mixed microorganism species that are mostly populations of lactic acid bacteria and yeast, photosynthetic bacteria, actinomycetes, and other types of bacteria (Borhan, 2011).

Vermicompost is an excellent soil amendment and bio-control agent which makes it the best organic fertilizer and more eco-friendly as compared to chemical fertilizers (Joshi et al, 2014).

Tomato is one of the high valued crops in the Philippines. Growing tomatoes using organic fertilization systems will lessen the use of synthetic fertilizers and that could improve the quality of tomato fruits, not just quantity. The study was conducted to evaluate the effects of vermicompost and different organic concoctions on the yield and quality of tomatoes.

The study aimed to assess the effect of different organic fertilization systems on the yield of tomatoes; and determine the quality of tomato fruits in

terms of ascorbic acid and beta-carotene contents as influenced by different organic fertilization systems.

This study is significant to researchers, students, and tomato planters. It contributes to the local studies on using organic fertilizers, especially the use of organic concoctions and the use of Indigenous Microorganisms. It also recommends local studies focusing not only on the increase in the yield of tomatoes but also on the fruit content in terms of ascorbic acid and beta-carotene. This would give insights into the production of organic tomato through the application of a different combination of IMO, FAA, FPJ, FFJ, and CalPhos as organic fertilizer. The study focused only on the yield and fruit quality of organically grown tomatoes using different fertilization systems. Vermicompost was used as basal fertilizers. It also used different organic foliar sprays such as IMO, FAA, FFJ, CalPhos, and FPJ.

MATERIALS AND METHODS

Site Description

The research was conducted from July 2017 to November 2017 at Escuala, Casiguran, Sorsogon. Casiguran is a 4th class municipality in Sorsogon Province, Philippines, at the south of Luzon island with coordinates of 12^0 83'N, 124^o 05'E. It belongs to Type 2 climate based on the Climate Map of the Philippines by the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA).

Being Type 2, Sorsogon has no pronounced dry season with pronounced rainfall from November to January. July-November average high temperature is 33^0C and the average low temperature is 25 ^{0}C. The average relative humidity is 45% - 90%. Soil Moisture is dry; the probability of rain is 50-90^0C. The type of soil present in Casiguran, Sorsogon is loam. The area was previously planted to bittergourd.

Soil Sampling and Analysis

Collection of the soil before planting was done by random sampling of ten soil samples in the entire planting area. The samples were mixed and air-dried. Analysis of soil sample for nitrogen (N), phosphorus (P), potassium (K), organic matter, and pH was done at the Soil Science Laboratory, University of the Philippines Los Baños. Soil analysis was done to characterize the nutrient status of the field. The amount of fertilizer applied was dependent on the result of soil analysis. Based on the result of the analysis, the soil has pH (5.1), a very high amount of OM (6.63%), a low amount of N (0.36 %), a medium amount of P (11 ppm), and a high amount of K (0.93 cmol/kg soil). The fertilizer recommendation on a hectare basis was applied to T1 (Farmer's practice) as follows: Basal fertilizer of three bags 14:14:14 + 1 bag of urea during transplanting; Side dress one month after transplant. One bag 0-0-60 + two bags urea; Top dress two months after transplanting per two bags of 0-0-60.

Test Crop

The variety of tomatoes used was Diamante Max F1. The seeds used in this study were procured from East-West, Philippines. It is a hybrid that is most suitable for cultivation in tropical lowlands. It displays strong vigor and wide adaptability to different growing conditions. It is resistant to bacterial wilt and heat tolerant variety (www.lazada.com.ph/east-west-seed-diamante-max-f1-tomato-kamatis-seed-11443496).

Experimental Design and Treatments

Randomized Complete Block Design (RCBD) was used. The treatments consisted of three replicates of the fifteen treatment plots. T1 was the Farmer's practice (control) and T2 to T15 were treated with vermicompost

(VC) as basal fertilizer. T3 to T15 had additional treatment of different organic concoctions as foliar spray: T2-Vermicompost (VC); T3-VC + Indigenous Microorganisms(IMO); T4- VC + Fermented Plant Juice(FPJ); T5-VC+ Fish Amino Acid (FAA) ; T6-VC + Fermented Fruit Juice (FFJ); T7-VC + Calcium Phosphate (Cal Phos); T8- VC + IMO + FPJ;T9- VC+ IMO+ FAA; T10-VC+ IMO+ FFJ); T11- VC+ IMO+ CalPhos; T12- VC+ IMO + FPJ + FFJ; T13- VC + IMO + FAA + FFJ; T14 -VC +IMO+FAA+CalPhoS; T15- VC + IMO + FFJ + CalPhos.

Statistical Analysis

Analysis of Variance was employed to determine the statistical significance of the treatments. LSD test was used to determine the difference between treatment means.

Cultural Management Practices

Preparation of Seedlings. Seeds were sown in previously prepared seedbeds (30 cm high, 1 m wide, and 10 m long). Vermicompost was added to the soil at the rate of 500 grams per square meter. A plastic roof was placed over the seedbed to protect the seedlings from heavy rains. Small furrows were set across the bed, 2-4 cm deep, and 15 cm between furrows. The seeds were sown a distance of about 2.5 cm (1 inch) along the furrows then covered with a thin layer of compost. The seedbed was covered with rice straw mulch. The mulch was removed after 4-5 days upon the emergence of seedlings.

Land Preparation. The soil was plowed and harrowed twice. Furrows were set at a distance of 75 cm.

Laying Out. The total experimental area was 1,377.87 m2 (37.75 m x 36.5 m). Plots were laid out following the treatments. The plot size was 6.75 x 3.5 meters. Distance between plots was 0.5 meters while the distance between replicates was 1 meter.

Transplanting. Healthy and uniform seedlings with 3-5 leaves each were selected. Transplanting was done four weeks after sowing at a distance of 50 cm between hills and 75 cm between rows. After transplanting, the soil was pressed gently around the base of the seedlings. It was watered before and immediately after transplanting. Replanting missing hills was done 5 days after transplanting.

Fertilization. Application of vermicompost and organic concoctions was done based on the treatments. Vermicompost analysis was as follows: N (1.44%), P (5.28 ppm), and K (0.06%). Recommended vermicompost was 5,555.55 ton/ha. For organic treatment from T2 to T15 vermicompost was placed 208 grams/ plant as basal fertilizer during transplanting. The different organic concoctions were applied on a weekly interval at the rate of 2.0 tbsp/liter of water.

Pest Control. The experimental area was surrounded by lemongrass to protect from insect pests. Also, Oriental Herbal Nutrient (OHN) was applied at a two weeks interval at the rate of 2.0 tbsp/liter of water.

Weeding and Watering. Both weeding and watering were done as necessary.

Trellising. A-Type bamboo trellis was placed one week before transplanting to support the stems of the plants.

Harvesting. Harvest started after 60 days from transplanting. Fruits were harvested at the breaker stage early in the morning. Harvested fruits were placed in crates lined with banana leaves or used newspapers to prevent mechanical damage to the fruits. Harvesting was done once only due to the limited fruits because of frequent rains and typhoons (PCAARRD Information Bulletin NO. 55/2015).

Data Gathered

Data were obtained from the fifteen sample plants that were used per treatment per replicate.

Yield Parameters

The number of fruits/plant – Total number of fruit per plant was counted manually.

Weight of fruits/plant (kg/plant) - Total weight of fruits per plant was measured using Metler top loading balance.

The number of marketable fruits/plant - Fruits with no damage were considered marketable and were counted.

Weight of marketable fruits/plant (kg/ plant) - Fruits considered marketable were weighed.

The number of non-marketable fruits/ plants - Defective fruits, which were damaged and considered non-marketable, were counted.

Weight of non-marketable fruits/plant (kg/plant) - All damaged fruits were weighed by using Metler's top-loading balance.

Quality of Fruits

Ascorbic acid - Samples (100 g) and beta-carotene content – Samples (100 g) of marketable tomato fruits and from the 1st harvest were brought to the College of Agriculture, Food Science Cluster, Food Chemistry Laboratory, University of the Philippines, Los Baños.

RESULTS AND DISCUSSION

Yield Parameters

Number and Weight of Fruits/ plant (kg). Fruit yield of tomatoes at different fertilization systems had significant differences in the number and weight of fruits per plant (Table 1) per plant. The T1-Farmer's Practice (synthetic fertilizer) significantly had the same number of tomato fruits with T2- Verrmicompost (Organic basal fertilizer) and with almost all of the other treatments with organic concoctions as foliar spray like T13, T5, T7-T15. The least number of fruits that were significantly lower than T1 were obtained from T4, T6, and T12.

The treatment with the highest weight of fruits per plant was at T14 (0.58), significantly the same with T13 (0.52), but statistically different with T1 (041) and other organic treatments from T2-T11 and T15. T1-Farmer's Practice (Synthetic fertilizer), T2 -vermicompost) and other treatments with foliar spray (T5 and T7-T11) were significantly similar in terms of the weight of fruits per plant and other treatments. The weights of fruits that were significantly lower than T1 were from T4, T6, and T12.

The number of fruits per plant in treatments with vermicompost and applied with organic concoctions spray was significantly the same with the farmer's practice using synthetic fertilizers. In terms of the weight of tomatoes per plant, it was observed that treatments with a combination of vermicompost, IMO, FFJ, and FAA and treatments with vermicompost, IMO, FFJ, CalPhos were significantly heavier than the treatment using Farmer's practice (synthetic fertilizer). Organic concoction mixtures of vermicompost + FFJ; vermicompost + FPJ; and vermicompost + IMO + FPJ + FFJ showed a significantly lower number and weight of fruits per plant compared with T1 (Farmer's practice).

Findings of this study are similar to the study of Joshi et al (2014) which said that vermicompost is ideal organic manure for better growth and yield of many plants.

Joshi and Vig (2010) found out that in using cattle dung vermicompost (VC), germination percentage was maximum at VC15 (soil + 15% VC). Almost all growth, yield, and quality

parameters increased significantly as compared to control; though the increase within the treatments was found not significant in number and heaviest weight of marketable fruits.

Average Number and Weight of Marketable fruits/plant. The number of marketable fruits among different fertilization systems had significant differences (Table 1). Specifically, T14 (15) with the highest number of tomato fruits per plant, was significantly different from the following treatments: T1-T4 and T6-T12. T14 was statistically the same with other organic treatments like T5 (14), T13 14) and T15 (13). T1 (Farmer's practice) was significantly the same with the number of marketable fruits in T2 (vermicompost) and other treatments with organic concoctions like T5 and T8-T11. Other organic concoctions like T3 (vermicompost + IMO) and T12 (vermicompost + IMO + FPJ + FFJ) had a significantly had a lower number of marketable fruits compared with T1.

Likewise, the weight of marketable fruits per plant had significant differences at different fertilization systems. T14 (0.54 kg) had the highest weight of fruits per plant. This was significantly the same with T13 (0.49 kg). T1 (.37 kg) compared with other organic fertilizer treatments showed a significant difference and had lower weight compared with T14 (.54 kg) and T13 (.49 kg). T1 also had significant difference but higher weight compared with T4 (.29 kg), T6 (.27 kg), T10 (.29 kg) and T12 (.22 g). Weight of fruits per plant at T1 had no significant difference on the following treatments: T2, T3, T5, T7, T8, T9, and T11.

The lightest tomato fruits per plant were at T12, but significantly the same with T4, T6, and T10. This is statistically different from the other treatments.

The composition of organic fertilizers that have a large number and heavier tomato fruits are the combination of vermicompost, Fish Amino Acid, Fermented Fruit Juice, and CalPhos. According to Alleje and de Villa (2014), vermicomposts are usually used as a soil conditioners. FAA is a source of nitrogen and contains an abundant amount of other secondary nutrients. CalPhos prevents blossom-end rot in tomatoes, prevents premature falling of blossom, improves fruit setting, and increases fruit production.

FFJ helps promote flowering and fruiting. It is a good source of potassium which speeds up the plant's absorption, resulting in sweeter tasting fruits. It helps maintain vigor in plants and resistance against pests. It adds to soil fertility and the advent of good colonies of microorganisms (Agricultural Training Center, 2006).

Average Number and Weight of Non-marketable Fruits/plant. The highest average number (3) of non-marketable fruits was obtained from T1 but not significantly different from T2, T3, T4, T5, T10, and T11 (Table 3). T15 had the lowest number of non-marketable fruits (1). Similarly, the weight of non-marketable fruits per plant showed that T15 had the lowest weight compared with other treatments and had no significant difference with almost all the other treatments except T10.

There was a lower quantity and lesser weight of non-marketable tomato fruits at T15. T15 was composed of vermicompost, fish amino acid, and Calphos. The implication is that these organic fertilizers produce good quality tomato fruits due to CalPhos that prevents blossom-end rot in tomatoes, prevents premature falling of blossom, improves fruit setting, and increases fruit production (Alleje and de Villa (2014).

Total Yield (t/ha). The highest weight of marketable fruits was 14.37

tons/hectare at T14, statistically similar to T13 (13.08 tons/hectare) and T15 (10.45 tons/hectare). This was significantly different from all the rest of the treatments and higher than the weight of tomatoes from T1 (9.94 tons/ hectare).

Organic fertilizer with the combination of vermicompost, indigenous microorganism, fish amino acid, and CalPhos gives higher biomass, increases fruit yield, increases marketable tomatoes, and lowers the number and weight of non-marketable tomatoes. Fish amino acid is a good source of nitrogen and contains an abundant supply of other secondary nutrients (Alleje and de Villa, 2014). Nitrogen is among the essential nutrients required by most crops in great amount. Nitrogen is necessary for chlorophyll production. Chlorophyll is needed to produce food for growth and nutrient uptake. It is also a component of amino acids, a building block of proteins. CalPhos is a good source of calcium and phosphate. Calcium helps induce flowering, prevents blossom-end rot in tomatoes, prevents premature falling of blossom, and improves fruit setting. Phosphorus is essential in root growth, development, and energy.

T14 had the best combination of nutrients that best support the growth and development of tomato plants (Table 1). While T13 organic fertilizer had a mixture of vermicompost, indigenous microorganism, fish amino acid, and fermented fruit juice. Fermented fruit juice is a good source of potassium that strengthens plants tissues, increases the size of fruits, increases crop resistance against diseases, and protects the plants from insects.

This result was comparable with the study of Zucco et al (2015) that compost was used as alternative fertilizer to reduce the need and dependence on synthetic, inorganic fertilizers. VC is a suitable tomato fertilizer for optimal growth at approximately 0.5-0.6 g/g added to the soil. It gives a similar growth rate of tomatoes using the standard inorganic fertilizer. Also, sandy soil with VC amendments generally increases tomato growth parameters compared to clay and loam soils, with loam generally providing the least.

Improvement in the weight of tomato fruits using VC and FAA in this study showed that vermicompost is ideal organic manure for better growth and yield of many plants. Application of vermicompost increases seed germination, stem height, number of leaves, leaf area, leaf weight, root length, root number, total yield, number of fruits/plant, chlorophyll content, pH of juice, micro and macronutrients, carbohydrates (%), and protein (%) content. There was an improvement in the quality of the fruits and seeds (Joshi et al, 2014).

Also, a similar result of this present study was cited by Verma et al (2015) that an increment of 31.83% in tomato yield was observed with the combination of Effective microorganisms (EM) compost and half the recommended dose of chemical fertilizers ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t/ha).

Quality of Tomato Fruits in terms of Ascorbic Acid and Beta-carotene

The ascorbic acid content of 1.58 mg/100 grams of fresh tomato fruits was observed with the following organic fertilizers treatment: T2, T5, T7, T10, T11, T12, and T14. Generally, those treatments with CalPhos have higher ascorbic acid (Table 2). This was followed by ascorbic acid content of 1.19 mg/100 g of fresh tomatoes at T4, which was higher compared with T1 (0.79 mg/100 g of fresh tomatoes). Also, some of the treatments of organic fertilizer had the same content of ascorbic acid with the T1 of 0.79 mg/100 g. These were T3, T6, T8, T9, T13, and T15.

Table 1. Yield of Tomato Fruits at Different Organic Fertilization Systems, Escuala, Casiguran, Sorsogon, August to November 2017.

Fertilization Systems	No. of Fruits/ Plant	Weight of Fruits/ Plant (Kg)	No. of Marketa-ble Fruits / Plant	Weight of Marketa-ble Fruits/ Plant (Kg)	No. of Non-Marketable Fruits/Plant	Weight of Non-Marketable Fruits/Plant (Kg)	Yield (t/ha)
T1 Farmer's Practice	15 ab	.41 b	13 b	.37 b	3 ab	.06 ab	9.94 b
T2 VC	13 bc	.41 b	11 bc	.38 bc	3 ab	.05 ab	10.13 bc
T3 VC + IMO	12 bc	.35 bc	10 c	.33 bc	3 ab	.04 b	8.84 bc
T4 VC + FPJ	11 c	.35 bc	11 bc	.29 c	3 ab	.08 ab	7.83 c
T5 VC + FAA	15 ab	.42 b	14 ab	.39 b	3 ab	.06 ab	10.41 b
T6 VC + FFJ	15 ab	.30 c	9 c	.27 c	2 b	.06 ab	7.11 c
T7 VC + CalPhos	13 bc	.38 b	12 bc	.35 bc	2 b	.05 b	9.27 bc
T8 VC + IMO + FPJ	12 bc	.39 b	12 bc	.36 bc	2 b	.04 b	9.68 bc
T9 VC + IMO + FAA	11 c	.41 b	12 bc	.38 b	2 b	.05 ab	10.18 b
T10 VC + IMO + FFJ	15 ab	.33 bc	11 bc	.29 c	3 ab	.09 a	7.68 c
T11 VC + IMO + Cal-Phos	13 bc	.37 bc	12 bc	.33 bc	3 ab	.07 ab	8.84 bc
T12 VC + IMO + FPJ + FFJ	11 c	.25 c	9 c	.22 c	2 b	.05 b	5.98 c
T13 VC + IMO + FFJ + FAA	16 ab	.52 a	14 ab	.49 a	2 b	.06 ab	13.08 ab
T14 VC + IMO + FAA + CalPhos	17 a	.58 a	15 a	.54 a	2 b	.05 b	14.37 a
T15 VC + IMO + FFJ + CalPhos	13 bc	.40 b	13 ab	.39 b	1 b	.02 b	10.45 ab

* Means having the same letter in a column are not significantly different using LSD.

Legend : CalPhos - Calcium Phosphate
IMO-Indigenous Microorganisms
FAA-Fish Amino Acid
FPJ-Fermented Plant Juice
FFJ-Fermented Fruit Juice
VC- Vermicompost

The ascorbic acid content in tomatoes in this present study ranged from 0.79 mg to 1.58 mg/ 100 g of fresh weight of tomatoes. This was very much lower compared to the results of other studies. The lower ascorbic acid content of tomato fruits may be due to the low temperature and frequency of rainfall during the whole period of their growth and development. The result in this study was parallel to the study of Liptay et al. (1986) proving that the ascorbic acid content of tomatoes was affected by temperature. The tomato fruit of the "Jumbo" cultivar, grown in the greenhouse was about 14 mg/100 g FW in June 1983 but increased to about 20 mg/100 g by mid-July. Tomatoes harvested in the greenhouse at the end of October had only 7 mg of AA/100 g FW. The temperature of the greenhouse was quite cool and certainly less than 20 ^{0}C during the latter part of October 1983. November, when the greenhouse was heated, the AA content rose to more than 11 mg/100 g FW. Tomatoes had lower quantities of AA when grown at low temperature compared to the quantities at higher temperatures (Liptay *et al*. 1986).

Abduli (2013) cited that vitamin C increased with the combination of 4:1 ratio of vermicompost and soil to 21.35 mg/100g fresh tomato. In this study, it appears that a combination of soil and vermicompost increases the amount of soluble and insoluble solids, total sugar, and vitamin C in tomatoes significantly.

Organic fertilizer at T15 had the highest beta-carotene content in tomato fruits with 47.80 mg/100g, followed by 24.19 mg/100g at T2, and 19.56 mg/100g at T14. Almost all the

Table 2. Ascorbic Acid Content of Tomato at different Fertilization Systems, Escuala, Casiguran, Sorsogon, August to November 2017.

Treatments	Ascorbic Acid (mg/100g)
T1 Farmer's Practice	0.79
T2 VC	1.58
T3 VC + IMO	0.79
T4 VC + FPJ	1.19
T5 VC + FAA	1.58
T6 VC + FFJ	0.79
T7 VC + CalPhos	1.58
T8 VC + IMO + FPJ	0.79
T9 VC + IMO + FAA	0.79
T10 VC + IMO + FFJ	1.58
T11 VC+ IMO + CalPhos	1.58
T12 VC + IMO + FPJ + FFJ	1.58
T13 VC + IMO + FFJ + FAA	0.79
T14 VC + IMO + FAA + CalPhos	1.58
T15 VC + IMO + FFJ + CalPhos	0.79

Legend : CalPhos - Calcium Phosphate FPJ-Fermented Plant Juice
IMO-Indigenous Microorganisms FFJ-Fermented Fruit Juice
FAA-Fish Amino Acid VC- Vermicompost

Table 3. Beta-carotene Content of Tomato at Different Fertilization Systems, Escuala, Casiguran, Sorsogon, August to November 2017.

Treatments	Beta-carotene (mg/100g)
T1 Farmer's Practice	11.06
T2 VC	24.19
T3 VC + IMO	13.78
T4 VC + FPJ	8.75
T5 VC + FAA	9.09
T6 VC + FFJ	17.32
T7 VC + CalPhos	16.71
T8 VC + IMO + FPJ	13.37
T9 VC + IMO + FAA	14.12
T10 VC + IMO + FFJ	18.20
T11 VC+ IMO + CalPhos	13.44
T12 VC + IMO + FPJ + FFJ	17.66
T13 VC + IMO + FFJ + FAA	15.41
T14 VC + IMO + FAA + CalPhos	19.56
T15 VC + IMO + FFJ + CalPhos	47.80

Legend : CalPhos - Calcium Phosphate FPJ-Fermented Plant Juice
IMO-Indigenous Microorganisms FFJ-Fermented Fruit Juice
FAA-Fish Amino Acid VC- Vermicompost

organic treatments were higher than T1, with 11.06 mg/100 g of beta-carotene.

The exception was T5, with 9.09 mg/100 g. The lowest content was 8.75 at T4 (Table 3).

This present study showed high content of beta carotene (8.75 mg to 47.8 mg/100 g) in tomatoes compared to other vegetables and other studies in tomatoes. According to Hignon and Norman (1996) as cited by Ahamad et al. (2007) beta carotene content in carrots was 10.110 mg/100 g, spinach 8.230 mg/100 g, lettuce 3.945 mg/100 g, and tomato 1.930 mg/100 g. There was a trace amount of beta carotene in onion, potato, and mushroom. Also, according to Ahamad et al. (2007), dark green vegetables contained more beta carotene as compared to other vegetables. Spinach contained 9.940 mg/100 g, followed by mint, kulfa, lettuce, and ladyfinger. All of these were all dark green in appearance. Apart from a carrot that contains 11.210 mg/100 g.

Organic fertilizer at T15 had the highest beta-carotene content in tomato fruits with 47.80 mg/100g FW and the lowest content of ascorbic acid with 0.79 mg/100 g fresh weight . Another organic fertilizer at T2 was highest in ascorbic acid content with 1.58 mg/100 g and second highest in beta-carotene with 24.19 mg/100 g fresh weight. The beta carotene content of tomato at different fertilization systems in this present study beta-carotene was very high compared to other studies, while the ascorbic acid content was quite low.

High beta-carotene content can be attributed to the increased nitrogen content of the soil as cited by Cherad et al. (2005). They found out that increasing N in the nutrient solution increased plant biomass, leaf tissue N, phosphorus (P), potassium (K), lutein-zeaxanthin, β-carotene, and chlorophyll. Very low ascorbic acid was due to lower temperature in the month of growing tomatoes, which was from August to October 2017. The planting

period was an off season for tomato growing. During this season typhoons are very common.

Alba et al. (2000) as cited by Othman et al. (2014) found out that temperature, light, mineral uptake, salinity, and irrigation have impact on carotenoid synthesis. An increased rate of potassium and phosphorus was found to enhance lycopene content of 20-30% in hydroponically grown tomatoes.

Also, as cited by Pertuzatti et al. (2015), ascorbic acid for organically grown yellow passion fruit was 2.3 x 102 mg/100g-1, In comparison, the conventional grown had 1.9 x 102. Carotenoid content for organically grown passion fruit was 13.99 mg/100 g while conventionally grown had 25.10 mg/100g.

CONCLUSIONS

This research showed that in terms of fruit yield the synthetic fertilizer used in T1 was comparable with the organic basal fertilizer of T2 (vermicompost). Among the different fertilization systems, it was T14 (VC+IMO+FAA+CalPhos) and T13 (VC+IMO+FFJ+FAA) which had more number fruits per plant and with the highest weight of tomato per plant. The highest average number of non-marketable fruits was obtained from T1 (Farmer's Practice). T15 (VC+IMO+FFJ+CalPhos) had the lowest weight of non-marketable.

Tomato fruits' ascorbic acid and beta-carotene contents were influenced by different organic fertilization systems. The highest ascorbic acid content was observed with the following organic fertilizers treatment: T2, T5, T7, T10, T11, T12, and T14. Organic fertilizer at T15 had the highest beta-carotene content in tomato fruits, followed by T2, and T14.

This study recommends the following: use this fertilization system on growing tomato during the dry season and study ascorbic acid and beta-carotene content of tomato fruit; apply this fertilization system using other variety of tomato; study the different growth, yield, tomato fruit ascorbic acid and beta-carotene content; and study the effect of T14 (vermicompost + fish amino acid + CalPhos) on the growth, yield, ascorbic acid and beta-carotene content on other the vegetables.

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ASIAN STUDENTS' PERCEPTION IN GENERATING INNOVATIVE IDEAS

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Abstract — Trends have rapidly overtaken the ability of businesses to evolve. Hence, the future industry would be needing more disruptive individuals to walk the speed of the industrial revolution. This paper aims to explore Asian students' behavioral skills towards developing an innovative idea. A series of exploratory personal interviews were conducted with twenty-two Asian students. The data were analyzed using a constant comparative method using the four innovative skills in the theory of entrepreneurial opportunity recognition. Using the theory's framework, four behavioral skills emerged during the process of developing innovative ideas for new ventures such as searching, experiential learning, exploration-based learning, and networking. Data revealed that the students could create new ideas through the learnings they had acquired from their interactions with diverse people during personal and curricular events. Their curiosity served as the primary reason why they delve into searching for more information using the four behavioral skills. Curiosity, therefore, was the primary antecedent of and catalyst for opportunity recognition and creation of innovative ideas. Another factor that motivated the respondents to search for answers was the desire to change the status quo. Finally, it was found that the Asian students were able to recognize some opportunities implied by the patterns of diverse information and ideas derived from the four behavioral skills.

Keywords — **Innovative skills, Asian students, opportunity recognition, disruptive innovation.**

INTRODUCTION

Future jobs are shaped by innovations and technology developments in the modern economy. The concept of Industry 4.0 is now globally taking place to achieve a higher level of operational productivity through automation (Ślusarczyk, 2018). Its key features are digitization, optimization, and personalization of production; automation and adaptation; human-machine interaction (HMI); cyber-physical systems; value-creation system as well as automated data exchange and communication (Roblek et al., 2016; Posada et al., 2015; Kovács and Kot, 2017; Veselovsky et al., 2018; Abbas, 2018; Mehdiabadi et al., 2020). However, Industry 4.0 is faced with challenges on the lack of digital exposure and training, leading to the shortage of qualified staff.

The tertiary students in this era belong to generation Z. They are the people who were born beginning in 1997. They have lived in a technological environment such that changes in behaviors, attitudes, and lifestyles have been observed from this generation (Dimock, 2019). Gen Z is also termed as Children of the Internet, Digital Generation, Digital Natives 78F, Media Generation.com Generation, iGen 88F, or Instant Online (Levickaite, 2010). Noticeably, they are characterized to have some form of reliance, freedom, individualism, addiction to technology, and speed. Hence, they are more adaptive to the changing trends. The unique trait in this generation is the technological advancement that prompts businesses to upgrade their processes (Berkup, 2014). They are the prospective movers and workers of future businesses that are envisioned to be technically sophisticated. The changing demands of the market bring such Prediction. Trends have rapidly overtaken the ability of businesses to evolve. Hence, the future industry would be needing more disruptive individuals to walk the speed of the industrial revolution.

This paper attempted to assess the perception of students in the Asia Pacific region in developing innovative ideas. More specifically, it determined their behavioral skills in creating opportunities that address the industrial revolution.

The study based its framework on the opportunity recognition theory of Dyer, Gregersen, and Christensen (2008) in generating novel ideas using four behavioral discovery skills such as questioning, observing, experimenting, and idea networking. Questioning is a mechanism for acquiring information leading to a new venture idea. It involves asking questions that challenged the current status and thinking of ways to make existing processes more efficient. Meanwhile, observing consists of utilizing multiple senses and is associated with compelling questions. It involves observing the environment and asking questions. Experimenting may refer to mental explorations, physical explorations, and tinkering with things as children and adults.

Meanwhile, idea networking means using social connections and interactions to generate opportunities as supplied by the set of inputs gathered. The tracing of behaviors of innovative entrepreneurs led the authors to understand the emergence of disruptive changes and innovations. Disruptive change is defined as severe surprises and unanticipated shocks that destabilize performance, even threaten ongoing viability. This theory was built by Christensen, which circles on the process of a new entrant with fewer resources being able to outperform an incumbent business. Its main characteristics are the initial inferiority on value attributes, new value proposition at lower prices, and the market entry from a niche (Schmidt & Junker, n.d.).

In a study on Students' Ideas on Innovations in Higher Education, the

role of innovative activity and participation in the creation, acquisition and application of innovations have become an essential factor in the academe (Jurgena and Cēdere, 2016). The shift to the industrial revolution triggers the shift to the education paradigm. The role of the academe is essential in the formation of competencies. In a formal learning environment where the mentor creates a culture of innovation, this further triggers the innovative behavior of the students (Roffeei et al., 2017). Students who are given tasks and are made to work independently are more likely to develop innovation skills (Martín et al., 2017).

Information searching was found to be an essential input in developing an innovative behavior. Zhong et al (2018) found a strong correlation between information-seeking behavior and innovation. Hence, to enhance a person's innovative behavior, the ability to acquire, process, and utilize must be cultivated (Yumin et al., 2017).

METHODOLOGY

The four behavioral patterns, such as questioning, observing, experimenting, and idea networking, were used to determine Asian students' innovative behavioral skills (Dyer et al., 2008). The respondents comprised two equally divided groups from the Philippines, Korea, Indonesia, and Japan, who belonged to the age group ranging from 20 to 41 years old. The first group consisted of eleven student attendees of the Asia Summer Program (ASP) 2019 held in Surabaya, Indonesia. The second group was composed of eleven Filipino students taking up BS Agri-Ecotourism Management Central Bicol State University of Agriculture (CBSUA), San Jose, Pili, Camarines Sur. The number of the local respondents was based on the figure of foreign respondents who participated in the survey to set a balance between the two groups. Participation was voluntary. The selection of the group from the ASP was made based on the premise that having Asian student respondents with different backgrounds would reveal more interestingly insightful representations of students' behavioral skills in developing an innovative idea. It was by chance that more female respondents participated in the interviews. Among the eleven (11) participants of the ASP, only two (2) were males. Meanwhile, in the local respondents, the participants who agreed to join the interview were all females. The exploratory interviews were conducted via face-to-face interaction.

The majority of respondents were in 4th-year college, taking courses in agriculture, tourism/agritourism management, education, business accounting, police administration, tourism and leisure management, and interior designing. Their family income per annum ranges from Php 30,000.00 to 1,100 000.00 (all currencies were converted in Philippine peso). Their family economic status perhaps implies why most of them were dependent on their parents for educational support. However, a few of them were fully self-supporting. Nine among the twenty-two respondents have already travelled to other countries, such as Malaysia, UAE, Thailand, Cambodia, Korea, Poland, Ireland, France, Czech Republic, Hungary, UK, Germany, Vietnam, Indonesia, China, Japan, Malaysia, Singapore, Taiwan, South Korea, Singapore, East Europe, and Hongkong. In contrast, the rest have not travelled to any country yet at the time of the survey.

The following questions were adapted from Dyer et al. (2008) to assess the perception of Generation Z across Asia in developing their innovative skills:

1. Tell us about the most valuable strategic insight/novel idea that you

2. In your opinion, do you have any particular skills that are important in helping you generate novel business ideas? Do you think the skills you have that have enabled you to be creative/ innovative (start an innovative business) are just part of your genetic makeup? Or do you think much of this ability was learned?

3. Are there any techniques you use or habits you have developed to help you come up with innovative ideas?

An exploratory interview was conducted with culturally diverse students to understand how they think of an innovative idea. Before the interview, the students were gathered and oriented on the purpose of this research. Each question was explained to them, and clarifications were correctly answered. Each student was asked as to their willingness to participate. Indeed, students willingly participated in the interview and were not forced in any manner. The data were analyzed using a constant comparative method of qualitative analysis. The socio-demographic variables earlier mentioned were considered in categorizing, comparing, and analyzing the data (SAGE Research Methods, 2005). The instrument's applicability to students was tested during the interview since a set of innovative entrepreneurs previously used this.

RESULTS AND DISCUSSION

Searching is one of the behavioral skills Gen Z Asian students used to recognize opportunities for new venture creation. However, only a few of them relied on this behavioral skill.

A 22-year old male Filipino student studying tourism management from Northwest Samar State University, Philippines said he usually reads motivational books and articles on current events. This probably gave him the idea to organize a motorcycle drag race in their locality, which is now an annual event.

The study revealed that one's strong desire to satisfy curiosity triggers this behavioral skill. Curiosity is a catalyst for information-seeking behavior to explore an environment and learn skills that might be useful in recognizing opportunities (Kidd & Hayden, 2016; Pathak, et al, 2017). Respondents' curiosity about things motivated them to delve into information which led to a discovery. Respondents in this study regularly spent time looking for and absorbing information by reading books, writing scholarly articles, watching educational television shows, watching DIY videos on YouTube, traveling, and hanging out with people that improve their information-searching skills. According to Pathak et al. (2017), curious people's insatiable demand for information motivates them to spend time searching and assimilating information by "listening to the music or news, browsing the internet, reading books or magazines, watching TV, movies, and sports, or otherwise engaging in activities not directly related to eating, reproduction, and basic survival."

A 21-year-old female Japanese student studying an international exchange program from Josai International University relates that if one pretends to be someone else, she can change her/his mindset and see things differently. This realization was made through her personal experience. According to her, the things that she usually does are reading books and studying. Moreover, she claimed she could memorize a lot given a short period.

Another respondent's behavioral skill is their ability to deduce and derive new ideas from their experience in the outside world. As cited in Jackson (2018), Oxford Dictionary defines experience as the "practical contact with and observation of facts or

events." Students learn formally and informally. Formal learning occurs in academic institutions, while informal learning takes place outside of schools and happens from student's exposure to and engagement in activities not done purposely for learning (Rogers, 2014 & Council of Europe).

Experiential learning means learning from experiences (Lehman, 2020), and new experiences motivate the conception of new ideas (Kolb, as cited in Mcleod, 2017). However, learning depends upon a person's ability to infer the gist implied by a particular situation (Rogers, 2014). This study discovered that Asian students had recognized opportunities and thought of new ideas from their experiences both from their schools and everyday life. According to Donaldson (2019) observation and empathy skills belong to the ten crucial soft skills of Gen Z. Soft skills are required for 4IR, along with workforce readiness, technical, and entrepreneurship. It refers to the "personal attributes, social skills, and communication abilities that support interpersonal relationships and interactions with others. It supports youth as they integrate and collaborate with internal and external workplace stakeholders" (Deloitte Global & the Global Business Coalition, n.d).

A 20-year-old female Indonesian student from Petra Christian University who studies interior designing said she always draws, brainstorms, makes diagrams, and then strolls around to refresh her mind. She claimed that through those activities, she could come up with new design ideas.

Observation skills are one's ability to acquire information from careful observation of things around him/her (Oxford Languages, n.d). On the other hand, empathy refers to the ability of a person to put oneself in the shoes of others (Morin, 2020). As a person observes his surroundings, his curiosity is stirred up. Questions begin to pop up in his mind, and he will soon search for answers (The Owl Teacher, 2016). As inquiry happens, learning also occurs, and the desire to improve the present status quo provides the impetus to figure out valuable ideas. This was accorded by the data gathered from the respondents of this study. According to them, they had thought of new ideas out of their curiosity and desire to change the current situation. A respondent recounted that when she had seen the pathetic condition of beggars living on the streets, she began thinking of how and why they arrived in that kind of situation. Then, the sympathy she had felt made her think of some ideas that could potentially change the unfortunate situation of beggars.

Then, a 20-year old female Korean student studying police administration from Dong Seo University shared that before, she had participated in a contest with no idea of what exactly it was. She just realized what it was all about after she had won the first prize. According to her, she has critical thinking that enables her to create novel ideas. Additionally, she said she can understand people through their body language and gestures. Finally, she loves playing escape games and counting as she feels through which she is intellectually improving.

Meanwhile, when respondents were asked about the things they regularly do, four habits emerged: watching television shows and videos on YouTube, traveling, and hanging out with friends. These habits exposed them to the outside world. Their experiences taught them practical lessons that could be used to recognize opportunities and think of innovative ideas for new ventures. These habits must have ameliorated their observation skills, social skills, and emotional intelligence, among others that enabled them to assimilate, interpret, and transform information

learned from their experiences into innovative ideas.

Another behavioral skill that respondents relied on generating innovative ideas was exploration-based learning or learning by Exploration. Exploration is an act of strategic investigation for the discovery of unknown things (thesaurus.com). It plays a vital role in active learning (Thrun, 1992). Exploration-based learning is a “learning approach that helps students learn through their curiosity and inquiry” (Verma, 2019). Curiosity is an intrinsic motivating factor for students to engage in an active investigation to discover strange things (Pluck and Johnson, 2011). In business, as shown by research, curiosity is significant for an organization's performance (Gino, 2018). As curiosity is triggered, employees think more critically and create more creative solutions (Gino, 2018). Generation Z is tagged as "digital natives" and presumed to be curious since they are more exposed to more information through social media than the generation before them.

Fedunik-Hofman (2019) found that generation Z where these students belong is said to be the "least curious generation" with a 66.5 curiosity index compared to the millennials with 72.2, making them the most curious generation. Nevertheless, the study discovered that the Asian students in this study were actively engaged in curricular activities. Their involvement must have seemingly led them to mental and physical exploration driven by their desire to seek information to satisfy their curiosity.

A 23-year old male Filipino student studying agricultural students from Camarines Norte, Philippines, realized that farming was a good source of income and business when exposed to it through learning sessions (classroom and laboratory). While the rest of the respondents said, they usually read books, browse the internet, watch videos on YouTube, stroll to different places, and hang out with friends.

Finally, the interview with Gen Z Asian students revealed that networking was also one of the behavioral skills they engaged in frequently, contributing to new ideas. Networking is an exchange of information and ideas through interaction among people of the same interest and field, either in social and formal gatherings (Kagan, 2020; Eid and Al-Jabri, 2016). Generation Z is a digital and social media native (Martis, n.d; Francis and Hoefel, 2018). Being so, they are “exposed to the internet, to social networks, and mobile systems” (Francis and Hoefel, 2018). Hence, they are assumed to be more into social networking than networking. Thus, they are more engaged in acquiring new information and ideas through social media. Aside from social networking, they were also actively involved in networking. They shared their social encounters, learnings, and skills that must have enabled them to think of new ideas.

A 21-year old female Indonesian student studying Business Accounting from Petra Christian University recounted that she thought of teaching people when she had discovered that their maid could not read. Her pity on her maid's illiteracy made her think of teaching people so that no more people would end in the same situation.

Another Indonesian student who is 22 years old studying Tourism and Leisure Management who had her internship in Taiwan said she learned how to interact with different people during her internship. Her sojourn and independence in Taiwan being away from her parents for some time taught her real-life lessons. She affirmed that having dealt with diverse people, she observed and realized that "when people are treated well, they will do the

new acquaintances. According to her, through her social interactions, she learns real-life lessons from the mistakes of others. Perhaps, her communication skills–being able to converse in different languages have enabled her to adapt to a new environment and blend with different personalities.

Networking nurtures students' communication and social skills. A Japanese respondent claims that the more people become exposed to something new, the more they learn and become adept. She can quickly adapt to a new environment, a new cohort of people, and new situations. She believes that she has probably developed this skill through her frequent transfer to different schools, which put her in a situation where she had no choice but to adapt herself to changes.

Academe serves as a training ground for students. This is where they are exposed to different curricular learning opportunities, tasks, and activities to hone their competence in a particular field. These learning activities allow students to work on tasks where they are encouraged to exchange ideas and learn from one another. The learnings derived from this interaction would influence their understanding of things. A student-respondent from Camarines Sur, Philippines, relates that she had thought of creating a trade fair when they had an extra-curricular activity in their school. The idea came up because she intended to conduct a fund-raising activity for the organization she belonged to. Through the trade fair she initiated, they were able to earn profit by selling personalized t-shirts. Nowadays, shopping is done online, where people do not need to go to a physical store. Instead of riding in a car or on a bus going to malls, what people need nowadays is just a gadget, internet connection, and of course, know-how in online searching. This new shopping fashion has resulted in the surge of a large number of online selling activities. Thus, excellent online selling skills are indeed an advantage for entrepreneurs. She is good at online selling, which probably means that she also has skills in online searching. Perhaps, this skill enabled her to come up with the idea of creating a trade fair and selling personalized t-shirts to earn profit for their organization. The bottom line is that the idea was generated through her engagement in an extra-curricular activity.

Meanwhile, a 23-year old Filipina studying BS Agriculture from Camarines Norte State College in the Philippines said their interactions with their professors and peers during their learning sessions had influenced their generalizations about their course. Moreover, it was inevitable that it has widened their understanding of the course and enabled them to recognize what opportunities they could push through after college.

Another Filipina student from the same school who is 29 years old said she invested in farming and she had had that idea because of her friend. She agreed to invest since she was knowledgeable in crop and livestock production and at the same time in management and in communications. Besides, she loves browsing the internet and chatting with her friends on social media by which she believed could further her knowledge of running a business. Considering all of these, she believed she could manage her investment well. It appears that family, friends, or peers influence important decisions in life. It is up to us whether to believe them or not. Trust and confidence will depend on the level of knowledge of the person involved. However, the opinion of the people who influences their decisions may matter. Similarly, another respondent from the same school was also encouraged by a friend to invest in a business.

Furthermore, to understand how it was really like managing a business, her friend let her run her saloon, where she said she learned so much. According to her, she is patient and loves to learn new things. Thus, she reads magazines, watches television shows, and attends seminars. These habits would help her to generate novel ideas she may need to progress her business.

These respondents usually come up with ideas through interactions with people around them. Their networking encounters mainly transpired during curricular activities. Students consider academe as their second home, where, aside from their community, they build and maintain most of their social networks, including their teachers and peers with whom they are associated 5-times a week. These people with whom they habitually associate themselves influence them directly or indirectly. Indeed, they came up with new ideas through the influence (encouragement and inspiration) of people around them.

There was a slight difference with the four behavioral skills that emerged from the study of Dyer et al. (2008), such as questioning, observing, experimenting, and idea networking. It was presumed that the slight difference might be attributed to the differences in the socio-economic characteristics of respondents who were unarguably from different generations. Dryer's study assessed the behaviors of the innovative entrepreneurs while this study focused more on Asian students.

Data revealed that the students in this study could create new ideas through the learnings they had acquired from their interactions with diverse people during personal and curricular events. It can be inferred that curiosity and the desire to change the status quo were the antecedents and catalysts for new idea generation. Moreover, networking enabled them to learn real-life lessons and the business endeavors to pursue after college. This context shows networking is another behavioral skill students engaged in to generate new ideas, and it mostly happened during their curricular activities.

CONCLUSIONS

The behavioral skills that emerged in this study appear to be almost similar to the behavioral skills in Dyer et al. (2008). Four behavioral skills that surfaced were *searching, experiential learning, exploring, and social networking* as significant to the acquisition of information that could be utilized to create novel ideas for new ventures. The slight difference may be attributed to the differences in the socio -economic characteristics of respondents who were unarguably from different generations. While the framework used by Dryer et al. (2008) used innovative entrepreneurs as respondents of their study, the present study employed the Asian students as respondents. Asian students could spot the opportunities implied by the patterns of diverse information and ideas derived from searching, experiential learning, exploration-based learning, and social networking. In other words, the respondents were able to recognize opportunities by integrating their associational thinking skills.

Curiosity motivated the respondents to delve into information and ideas through the four behavioral skills in this study. In other words, curiosity was the primary antecedent of and catalyst for opportunity recognition and innovative ideas that address the industrial revolution. Aside from curiosity, the desire to make things better (desire to change the status quo) also motivated them to think of innovative ideas that address the call for industrial revolution (4IR).

With these findings, the traditional method of teaching such as the lecture and classroom-type of exercises may no longer adapt to their current behavior. Academic programs may integrate a longer period for industry engagement so that the skills on searching, experiential learning, exploring, and social networking may be harnessed and used before they graduate from their respective programs.

Finally, while this study focused on identifying the curiosity factors, it did not address the motivational factors such as monetary, psychological, and social gains, which can trigger the associational thinking behaviors of the students. Further study may also be conducted comparing entrepreneurial and non-entrepreneurial backgrounds of students in developing innovative ideas.

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MORPHOLOGICAL AND PHOTOSYNTHETIC RESPONSES OF IN VITRO CULTURE OF INDIA ECHINACEA (*Andrographis paniculata*) TO LIGHT SPECTRUM AND CARBON SOURCES

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Abstract — *Andrographis paniculata* (Burm.F.) Wall ex. Nees is an important medicinal plant in ASEAN countries that drives a high demand in the worldwide medicinal plant trade due to its andrographolide content. To find out optimum lighting and culture conditions *in vitro*, seed cultures were grown on photoautotrophic (sucrose- free) and photomixotrophic (3% sucrose) media. Blue and red monochromatic LED lights were used at an irradiance intensity of 40 µmol m^{-2} s^{-1} for 20 days as an energy source. Photosynthetic responses were measured in terms of chlorophyll content and net photosynthetic rate. Morphological responses were measured in terms of leaf and internode count, shoot length, number of roots, root length, fresh weight, and dry weight. Results were analyzed using SPSS 11.5 software. Gas chromatography results show that net photosynthetic rate was higher under blue light while red light resulted in taller plants. Root count and root length were higher under red light by 11% and 9.7% respectively. Sucrose reduced the net photosynthetic rate under both light spectra. Furthermore, plants under photoautotrophic conditions grew taller shoots and higher fresh weight. However, photomixotrophic blue light plants showed the highest dry matter content. Spectrum- wise, red light plants have a higher dry weight but when sucrose was involved in the growing medium, blue light plants outweighed it by as much as 7.91%. This suggested hyperhydric tissues in other cultures. Photosynthesis is constrained often by the low concentration of CO_2 in photomixotrophic cultures as sucrose has been observed to act as an osmotic agent in *in vitro* culture systems.

Keywords — ***Andrographis paniculata*, photosynthesis, red light, blue light, sucrose**

INTRODUCTION

Photosynthetically active radiation (PAR) drives the photosynthetic process in a spectrum-specific effect. Blue and red monochromatic lights have effects both on photosynthesis and morphogenesis and are the more photosynthetically effective spectra (McCree,1972; Inada, 1976). Among the effects of red light is stem elongation (Yanagi,1996) while blue light favors chlorophyll formation (Humbeck et al., 1984) and more efficient photosynthesis. Nearly all of the carbon and chemical energy needed for plant growth is provided during photosynthesis (Björkman, 1981). However, plants can also utilize soluble sugars as a source of carbon. Hdider et al (1993) reported the photosynthetic influence of sucrose in plants. Sucrose also influenced in vitro plant organogenesis (Arigita et al., 2002).

These findings are the fundamentals in investigating the effects of the light spectrum and sucrose as carbon sources on the medicinal plant *Andrographis paniculata. A. paniculata* (Burm. f.) Wall ex. Nees is an important medicinal plant in ASEAN countries (Akbar, et. al., 2011; Kabir, et al., 2014) that drives a high demand in the worldwide medicinal plant trade (Hossain et al., 2014) due to its andrographolide content (Shah et al., 2007). *In vitro* culture of this plant could result in maximizing andrographolide production.

However, optimum conditions for effective in vitro production must be known thus, the effects of red and blue light and sucrose as a carbon source on *Andrographis paniculata* were examined.

MATERIALS AND METHODS

Plant Materials and Treatments

Prior to germination, seeds of *A. paniculata* were surface-sterilized with 20% Clorox (The Clorox Co., Oakland, CA, USA) and mechanically shaken for 20 minutes. Surface- sterilized seeds were then grown on Murashigue and Skoog (1962) medium under white fluorescent light before treatment application. The culture medium was pH- adjusted to 5.7 before autoclaving. Thirty-day- old plants were then aseptically transferred to Phytagel-solidified MS medium inside 250 mL glass vessels for photoautotrophic and photomixotrophic growth. Photomixotrophic medium contained 3% sucrose as a source of carbon. Ambient CO_2 was utilized as a carbon source for photoautotrophic growth. The lid of photoautotrophic vessels was perforated and covered with gas-permeable microporous polypropylene film (0.22 µm pore size) to allow air exchange. Monochromatic blue and red LED lights were used to subject the plants under different PAR. LED lights were set at 40 µmol m-2 s-1 of photosynthetic photon flux (PPF) for 20 days.

Morphology Parameters, Fresh and Dry Weights

A number of leaves were counted during days 0, 10, and 20 of the experiment. The final leaf count was done on day 20. Shoot length was measured from the crown of the plant up to the terminal bud. The shoot and root length of the test plants were measured after taking them out of the growing vessel. The length was measured from the belowground base of the plant up to the tip of the primary root. Shoot length was measured on days 0, 10, and 20 of the experiment. The number of internodes was counted through visual recognition on day 20.

Fresh weight of the whole plant was measured using an Adventurer OHAUS ARC 120 2- point balance. Plants were removed from the growing bottle and cleaned off of residual MS medium that attached to the plant roots. The dry weight of the whole plant was measured using an Adventurer OHAUS ARC 120 2- point balance. The whole plant parts used for measuring fresh weight were cut into stem, leaf, and root components before being put into aluminum foil packets. Samples were then oven-dried using Hot Air Oven at 65° C for 72 hours. Samples were then allowed to cool at room temperature before being weighed.

Net Photosynthetic Rate and Chlorophyll Content

The net photosynthetic rate was measured by reference to the internal and external concentrations of CO_2 within and outside the growing bottles. This was quantified through gas chromatography using Shimadzu GC Model GC- 17A. The net photosynthetic rate was then computed using the formula of Fujiwara, et. al., (1987) given as [Pn] = K x E x V (Cout - Cin)/ Leaf Area; where K is a conversion factor converting CO_2 amount from volume to mole (40.5 mol m-3 at 28° C) E is the number of air exchanges per hour (2.32 h-1) and V is the air volume of the growing vessel (0.0025 m3) (Fujiwara et al., 1987). Non- destructive chlorophyll measurement was done using SPAD 502 Chlorophyll Meter. Measurement was taken on the first or second leaf of the sample plants by attaching the probe onto three different sections of a leaf. The average of the three readings is interpreted as the instantaneous chlorophyll content.

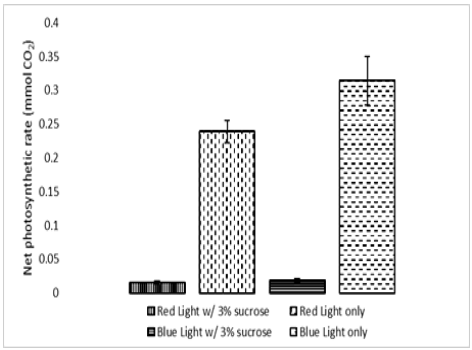
Data Analysis

Data were analyzed using SPSS Version 11.5. The data were run through a Two-Factor Analysis of Variance (ANOVA). Significant differences among means were calculated through Duncan's Multiple Range Test.

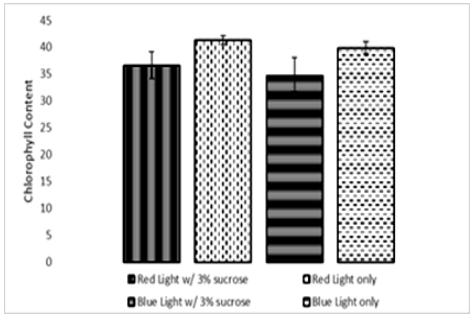
RESULTS AND DISCUSSION

The net photosynthetic rate was lower among photomixotrophic vessels (Figure 1-a). Plants in this culture suffered as much as a 94% reduction in their net photosynthetic rate regardless of the light spectrum. It was observed that leaves under photomixotrophic culture showed more folds and curls. Spectrum-wise, red light plants assimilated 13-17% lower while blue-light plants showed relatively higher assimilation. Moreover, regardless of cultural conditions, blue light plants show to be more photosynthetically efficient than the red- light counterparts. Although chlorophyll content data (Figure 1-b) might show red-light plants to probably have more roots that light-harvesting complexes (LHCs), variance analysis shows no significant chlorophyll content difference. Chlorophyll content, however, was 16% higher in red-light photoautotrophic conditions and 5% higher in red-light photomixotrophic conditions than on the blue light cultures.

Number of internodes was not affected (Figure 2-a) but showed to have possible direct correlation to shoot length. (Figure 2-b). However, shoot length was affected being taller under photoautotrophic conditions. Although insignificant, an inverse relationship was observed where under photoautotrophic conditions, blue light triggered a 7% increase in shoot length but in photomixotrophic conditions, red light plants were 13% taller. Root length (Figure 2-d) and the number of roots (Figure 2-c) were affected by red light. There were 11% more were 9.7% longer on red light plants. Although insignificant, a spectrum-restricted observation was noted on red light



1-a



1-b

Fig. 1. Photosynthetic responses of *Andrographis paniculata* grown under photomixotrophic and photoautotrophic condition *In vitro*; net photosynthetic rate (Figure 1-a) and chlorophyll content (Figure 1-b).

plants where the red light-triggered increase in root count under photomixotrophic conditions. On the other hand, red light promoted longer roots under photoautotrophic conditions. Unlike blue light where its effects were only on shoot length, red light encompassed both root count and root length.

In terms of weight, fresh weight (Figure 2-e) was higher among red light plants than those grown on blue light. It was lowest on photomixotrophic blue light conditions and highest on photoautotrophic red light conditions. However, photomixotrophic blue light plants showed the highest dry matter content. Spectrum-wise, red light plants have a higher dry weight but when sucrose was involved in the media, blue light plants outweighed it by as much as 7.91%.

Plant development and physiology are strongly influenced by the light spectrum of the growth environment (Hogewoning et al,2010) and different wavelengths in the PAR have specific effects on the plant. For in vitro cultured *A. paniculata*, net photosynthesis was highest among plants grown under blue light in sugar- free medium. Blue light is involved in a wide range of plant processes such as phototropism, photomorphogenesis,stomatal opening and leaf photosynthetic functioning (Whitelam and Halliday, 2007). Moreover, blue light has been associated with the expression of 'sun-type' characteristics such as a high photosynthetic capacity at the chloroplast level, (Lichtenthaler et al., 1980).

A lower photosynthetic rate under red light has been observed in many plant species (Matsuda et al., 2004; Goins et al., 1997) than those plants grown under the presence of blue light. Long-term exposure to monochromatic red light can result in photosynthetic dysfunctional leaves characterized by a suboptimal and heterogeneously distributed dark-adapted Fv/Fm, a stomatal conductance unresponsive to irradiance, and a relatively low light-limited quantum yield for CO2 fixation (Hogewoning et al., 2010). Photosynthesis is constrained often by the low concentration of CO2 in photomixotrophic cultures (Kubota, 2002). The decrease in Pn could also be attributed to lower chlorophyll content. Mohamed and Alsadon (2009) showed that the total chlorophyll content was higher when plants were grown in ventilated vessels than in non-ventilated vessels.

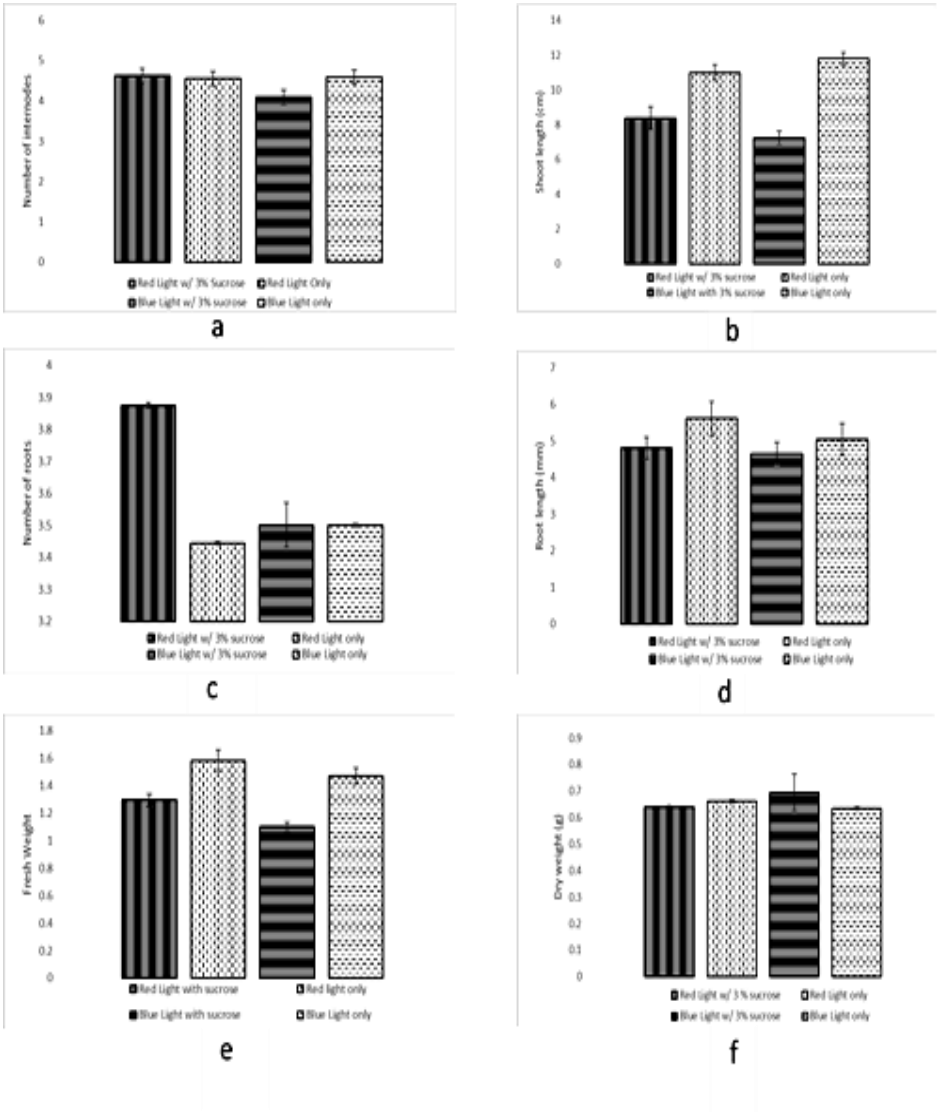


Fig. 2. *Andrographis paniculata* cultured on photomixotrophic and photoauto trophic conditions under blue and red light. (Fig. 2a. Number of inter nodes; Fig. 2b. Shoot length; Fig. 2c. Number of roots; Fig. 2d. Root length; Fig. 2e. Fresh weight, and Fig. 2f. Dry weight).

Red light has been frequently shown to affect plant morphogenesis (Fukuda et al., 2011) which stimulates shade avoidance response in plants such as enhanced stem elongation (Smith, 1995). Highest shoot length was observed on plants grown under red light which coincides with this finding but it does not differ significantly with those grown under blue light. Blue light irradiance has been found to inhibit hypocotyl elongation (Hoenecke et al., 1992) and the reduction of blue light increased shoot length (Mortensen and Stromme, 1987) while red light irradiance resulted into longer shoots (Fukuda et al., 2011). Chlorophyll plays a critical role in the process of

photosynthesis. Changes in its level have been used to evaluate photosynthetic activity and changes in the proportion of chlorophyll *a* to chlorophyll *b* have been used as a marker for tolerance to abiotic stresses in plants (Larcher, 1995). Hassankhah et al, (2014) showed that plants grown in ventilated vessels had significantly higher chlorophyll content. The exogenous supply of sucrose which is not required for the normal development of photosynthetic apparatus normally produces low chlorophyll content in *in vitro* plants (Grout and Donkin, 1987; Mohamed and Alsadon, 2009) but this decrease is not significant as was observed by Cui et al., (2000). Although blue light has been reported to likely have more chlorophyll content (Hogewoning et al., 2010), the red light spectrum has been shown to induce more chlorophyll pigments in *Andrographis paniculata.*

Root formation is influenced by red light (Gabryszewka and Rudnicki, 1997). However, this result was only obtained when the red light is applied in combination with an exogenous auxin (Rossi et al., 1993). The results of Rossi et al. (1993) could be explanatory of the findings in this study that neither red nor blue light spectrum resulted in higher root length. However, Rossi et al. (1993) have observed that red light could influence root formation when in combination with auxin. This could explain why sucrose-treated plants under red light showed the highest average number of roots. Sucrose has been reported to have caused possible hormone-like effects in shoot cultures of potatoes (Vinterhalter et al., 1996). Taylor and van Staden (2001) found that *in vitro* cultured *Eucomis autumnalis* dry weight was highest in photomixotrophic cultures with 4% sucrose. The absence of osmotic stress among plants grown in a photoautotrophic medium could have encouraged hyperhydricity resulting in low dry weight. Lower dry weights of the hyper-hydrated shoots could be attributed to the high water content of the shoots (Afreen, 2007). Blue light caused a higher number of internodes contrasting the findings of Lund et al, (2007), stating that the number of internodes was determined by the red to a far-red ratio of light. But when exogenous sucrose is involved, *A. paniculata* tends to have a higher number of internodes.

In terms of fresh weight, several previous findings on *in vitro* cultures were confirmed by the observations in *A. paniculata*. In the studies of Kozai et al. (2002) and Rahaman and Alsadon (2007), photoautotrophic cultures showed higher fresh weight than those in photomixotrophic cultures. However, on a dry weight basis, photomixotrophic blue light plants were heavier. This could suggest hyperhydric tissues in other cultures. Sucrose has been observed to act as an osmotic agent in *in vitro* culture systems (Mukherjee et al., 1991).

CONCLUSIONS

Growing seed cultures on photoautotrophic and photomixotrophic media were done to find out the optimum lighting and culture conditions in vitro. The energy source made use of Blue and red monochromatic LED lights at an irradiance intensity of 40 µmol m-2 s-1 for 20 days. Photosynthetic and morphological responses were measured and analyzed using SPSS 11.5 software.
The net photosynthetic rate was higher under blue light based on the gas chromatography results. Likewise, exposure of plants to red light produced taller plants. More roots (11%) and longer roots (9.7%) were produced under red light. Under both light spectra, sucrose was found to reduce the net photosynthetic rate. Plants were found to have taller shoots and higher fresh weight under photoautotrophic conditions.

Photomixotrophic blue light plants had the highest dry matter content. The red light plants have a higher dry weight however it was outweighed at 7.91% by the blue light but when sucrose was in the growing medium suggesting the presence of hyperhydric tissues in other cultures. Low concentration of CO_2 constrained photosynthesis in photomixotrophic cultures with sucrose observed to act as an osmotic agent.

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STINGLESS BEES (HYMENOPTERA : APIDAE: MELIPONINI) UNDER GENUS *Tetragonula* in the PHILIPPINES

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Abstract — The nesting habits, morphological and genetic structures of stingless bees were assessed using the traditional taxonomic characters and BLAST analysis results in 18 populations of *Tetragonula* in the Philippines. Different structures, shapes, and colors of stingless bee entrances were recorded. Dendrogram was drawn using the measurement derived from the morphometric characters. The majority of the species build their nest in tree cavities, cemented walls, and rocks with circular-shaped entrances. Irregularity in shapes of colonies from Ogbong (Catanduanes), Calapan (Oriental, Mindoro), Alabat, and Perez (Quezon) was observed. Most colony entrances are hard and dark in color, facing east with three to 15 guard bees. Light-colored entrances were observed in colonies from Cacilles (Hernani, Samar) and Pangi (Gasan, Marinduque). Nesting habits of stingless bee species were not species-specific. The only amplicon from 16S rRNA gene reproducible in 12 colonies was analyzed. No hits found in sampled specimen from Panim-an, Caramoan, Camarines Sur, and Oriental, Mindoro (Calapan and Naujan), but, samples from Tagbilaran, Bohol, and Tagum, Davao was confirmed. In Luzon, the identified species of stingless bees were *Tetragonula iridipennis* found in Alabat and Perez (Quezon), Gasan (Marinduque), Garchitorena (Camarines Sur), Viga (Catanduanes), Placer (Masbate) and Naujan (Mindoro) while *T. laeviceps* were found in Calapan (Mindoro), and Caramoan (Camarines Sur). For Visayas, the identified species were *T. laeviceps* in Hernani (Samar), Marbuena, and Nasidman (Iloilo) while *T. sapiens* in Tagbilaran (Bohol). In Mindanao, the species was identified as *T. sapiens* in Tagum (Davao). An unidentified species was found in Paniki (Aroroy, Masbate).

Keywords **— Stingless bees, colony characterization, *Tetragonula* species gene sequencing.**

INTRODUCTION

The tribe Meliponini (stingless bees) are eusocial insects (Michener and Sakagami, 1990), which means they exhibits reproductive division of labor, cooperative brood care, and overlap of generations exhibits a pantropical distribution (Francisco et al., 2001) and are far most diverse, morphologically and behaviorally (Michener, 2013).

During explorations of the tropical world, stingless bees enjoyed a long history of discovery of many species which have been identified and described (Rasmussen, 2009). Species vary considerably in their nest architectures, which range in design for brood cells arranged in horizontal or clusters, constructed within crevices of trees, walls of human buildings, limestone cliffs, or even in the ground (Roubik, 2006; Michener, 2007, 2013; Banziger et al.,2011).

For centuries, bees made a huge impact on people worldwide, providing food (pollen and honey) and medicine (propolis) to cure a variety of ailments (Bankova, 2005). Stingless bees play an important ecological role as effective pollinators of many plant species, both wild and cultivated, and seem to be good candidates for future applications as commercial pollinators (Amano et al., 2000).

However, despite their ecological and economic benefits, limited studies have been conducted regarding the diversity, distribution, genetic classifications, and morphological variations of this group of insects. Therefore, in order for the species to complete its information and understand effectively and efficiently their habits and behaviors, it requires strongly supported studies on their nesting habits and behavioral variations by assessing its morphological and genetic response relevance (Fernandes-Salomão et al., 2002; Costa et al., 2005; and Cruz et al., 2006). The same is true in the Philippines.

Thus, the study aimed to assess the nesting habits, morphological, and genetic differences among and between the stingless bee populations occupying different island locations in the Philippines.

Information on morphological, behavioral, and genetic variation are of great importance to understand the phylogeographical patterns of the species. These informations are also relevant for the development of effective conservation strategies and to help stingless bee enthusiasts and beekeepers in selecting the best species of stingless bee for honey, pollen, and propolis production. Likewise, the results obtained in this study will help them improve techniques in colony propagation, queen rearing, drone rearing, and, possibly, artificial insemination. This research also enabled us to uncover more of the mysteries of these unique bees, and to develop their potential.

MATERIALS AND METHODS

Collection of Stingless Bee

Samples of the stingless bees were collected in 10 provinces in different islands of the Philippines. Four samples were collected from the province of Camarines Sur (Caramoan and Garchitorena , two individual feral colonies found from the islands of Masbate (Aroroy and Placer), Iloilo (Marbuena and Nasidman), Mindoro (Calapan and Naujan), Quezon (Perez and Alabat) and Gasan, Marinduque, and one individual nest from Hernani, Samar; Tagum, Davao; Tagbilaran, Bohol and Viga, Catanduanes.

The tropical climate was noted in all provinces. This type of climate was initially described as the most preferred temperature by the stingless bees

(Ramirez et al., 2010; and Solórzano-Gordillo et al., 2015). Likewise, different types of vegetation were recorded in different provinces. Majority were planted with rice (Quezon; Davao; Camarines Sur, Masbate, Mindoro, Samar, Bohol, Marinduque and Catanduanes), coconut (Quezon; Davao; Camarines Sur, Masbate, Mindoro, Marinduque and Catanduanes), banana (Davao, Samar, Bohol and Masbate), mango (Samar, Quezon, Camarines Sur, Catanduanes, and Masbate), Lanzones (Davao and Quezon) and flowering plants (Marinduque). The types of vegetation play a significant role in the formation of stingless bee colonies which are all good sources of food for the stingless bee except for rice.

Collection of Stingless Bees

Twenty-five (25) adult workers of stingless bees in every feral colony found in an area were collected, for a total of 300 samples. Stingless bees were caught by direct sampling at the entrance of their nests either by picking them one by one using forceps or by catching them using an insect net. Collected adult bees were placed in vials and preserved in 95 percent ethyl alcohol and labeled properly and set aside for morphometric study and DNA sequencing.

Voucher specimens from all sampled localities were added in the stingless bees collection of the Project on Bio-Informatics of Stingless Bee which was funded by the Department of Agriculture-Bureau of Agricultural Research (DA-BAR).

Morphometric

Morphological examination of stingless bees workers was done using the Stereoscopic Microscope (1X & 4X magnification), fitted with an ocular micrometer. Thirty-one (31) morphometric characters (19 individual characters, 12 ratios) were recorded. These parameters were adopted from the works of Sakagami (1978) and Dollin et al. (1997). Nineteen of which are from the head, malar area, gena, compound eye, ocelli, right antennae, right forewing, hind tibia, and hind basitarsus.

Morphometric Statistical Analysis

The results of morphometric studies were subjected to Stepwise Discriminant Analysis (SDA) using SPSS version 21 with p=0.05 for inclusion of a variable and p=0.10 to exclude a variable then subjected to Canonical Discriminant Analysis.

In order to show the variability of the most important quantitative characters for each species in a clear and readily comparable way, graphical tests in the form of scatter plots that illustrate the variation in parameters were applied.

Euclidean distances among populations were calculated and subjected to single linkage clustering (Nearest-Neighbor) using similar software (SPSS ver. 21) in order to construct a dendrogram that would reflect the inter-group morphometric similarities of species.

Gene Sequencing

Collected bees stored in alcohol served as biological replicates. Individual bee from each replicate was washed thrice with distilled water and dried on paper towels. The bees were placed in 1.5 mL tubes and DNA extraction was performed using the DNeasy Blood and Tissue Kit (Quiagen) following the manufacturer's protocol. The eluted genomic DNA was analyzed by agarose gel electrophoresis in a 1.3 percent agarose gel run for 30 minutes at 100V in the labnet gel X1 Ultra V-2 electrophoresis apparatus. Then, it was visualized using the alpha Innotech

Alphalmager MINI.

Extracted genomic DNA was used as a template for PCR using the Invitrogen TM PCR Super Mix. Each reaction consisted of 45 uL of the supermix, 3 uL template DNA, and 1 uL each of the forward and reverse primers.

The primers used (forward and reverse) were specific for the mitochondrial 12s (5'TAC TAT GTT ACG ACT TAT 3' and 5'AAA CTA GGA TTA GAT ACC C 3') and 16s rRNA (5'TTA CGA TGT TAT CC TAA 3' and 5' CGC CTG TTT ATC AAA AAC AT 3'), cytochrome oxidase I (CO1) (5' TGA TCA AAT TTA TAA T 3' and 5' GGT AAA ATT AAA ATA TAA ACT TC 3'), cytochrome oxidase II (COII) (5' ATT AGA TGT TGA TAA TCG 3' and 5'ACA AAT TTC TGA ACA TTG 3'), or the NADH dehydrogenase (NADDH) (5' CTA AAG TTG ATG AAT GAA CTA AAG 3' and 5' GCT CAT GTT GAA GCT CC 3') gene fragments.

PCR was done using the Veriti 96 well Thermal Cycler (Applied Biosystems). PCR products were checked for amplification by subjecting them to agarose gel electrophoresis in a 2% agarose gel run at 100 V for 30 minutes. Successfully applied gene fragments were sent to 1st BASE DNA Sequencing Services for single-pass capillary sequencing.

Basic Local Alignment Search Tool (BLAST) analysis was used to determine the similarities and relationships between and among species.

Identification and Confirmation of Results

Confirmation of the species of the stingless bee samples collected was facilitated by the results of the morphometric study and the genetic sequences with the help of different books, catalogs, and taxonomic keys provided by many authors. If confusion arises, the nest characters were considered to identify the species. After the bees have been identified, these were plotted into a map using the Geographical Information System. The map shows the locations and the species of the stingless bees collected in the different islands of the Philippines.

RESULTS AND DISCUSSION

Species Identified using Nesting Sites and Hive Entrances

Nesting Sites of Stingless Bees. A total of 18 colonies of stingless bees were collected and used in the study. The majority of these colonies were collected in Luzon (13 colonies), Visayas (4 colonies), and Mindanao (1 colony). All colonies were found in hard, solid, and permanent objects like in crevices of the living tree trunk, decaying logs, cemented wall, and rocks, whereas others were found in some parts of human houses, such as wall crevices, drawers, and bamboo cliffs. In particular, each stingless bee showed specific nest requirements according to its habitat quality.

It was found that in Luzon, the highest number of colonies were found living in cavities of tree trunks and walls of buildings, followed by colonies living in crevices of rocks and decaying logs. In Visayas, nesting sites of colonies were in tree trunks, dead logs, buildings, and crevices while the colony from Mindanao nested inside an old and damaged table. The study revealed that nesting habits of stingless bees found in Camarines Sur (Caramoan and Garchitorena), and Masbate (Placer) were recorded living in a cavity of *Avecennia alba* (myiapi*)*. Likewise, a colony from Tagbilaran, Bohol was found similar in nesting habits in colonies found in Masbate, Marinduque, Oriental Mindoro, and Quezon. All these colonies were recorded living in crevices of building walls. Likewise, three colonies were

found to live in stone crevices in Mindoro, Marinduque, and Camarines Sur.

***Nest Entrances of Stingless Bee Colonies*.** Different shapes, colors, and rigidity of nest entrances of stingless bees were observed. Most commonly, tubular, ovular, round-ringed, and irregular entrances were found in different islands in Luzon. Similar shapes of entrances were recorded in Camarines Sur (Caramoan and Garchitorena), Iloilo (Marbuena and Nasidman), Bohol, and Marinduque, with round and tubular types. However, in the islands of Quezon and Catanduanes, irregular entrances were recorded.

The color of their entrances varied from light brown to black (Figure 1-3). Light-colored entrances, however, were observed in Camarines Sur, Iloilo, Samar, and Marinduque, while dark colored entrances were observed in colonies collected in Davao, Bohol, Quezon, and Camarines Sur. All entrance holes, on the other hand, were observed facing east with 3 to 15 bee guards.

It was recorded that all colonies collected in Visayas and Mindanao islands were characterized by soft rigidity. A similar feature was observed in six colonies collected in Luzon (Camarines Sur, Mindoro, Catanduanes, Marinduque, Masbate, and Quezon).

Based on the nesting sites of colonies, the identified species belong to the *Tetragonula* group. Since most of the species were cavity nesters, these species belongs to *laeviceps* or *iridipennis* group. This study corroborates with the results of Gajanan et al. (2005) which recorded nesting sites of *T. iridipennis* in crevices of tree trunks, stone and mud walls, corners of walls, and termite mounds. This further corroborates with recent studies in the Bicol region by Davila , 2017; Penaverde , 2017; and Esplana ,2017 that the cavity nesters found in different areas in the region were identified as *T. iridipennis*, *T. laeviceps*, *T. sapiens*, and *T. fuscobalteata*.

Species Identified using Morphometrics

***Morphological characteristics*.** The workers of each bee species have varied morphological characteristics. Out of 31 morphological characters analyzed, 27 of which were identified by the stepwise analysis as significant variables that had considerable contributions to the intra- and interspecific separation of the group samples. Seventeen for individual characters such as HW, LOD, SC, BL, GW, F4W, HTL, ML, WL2, MOD, IOD, OOD, F4L, HTW, EW, EL, and HBW) and 10 for ratios (SC/EL, ML/FW, HTW/HTL, IOD/OOD, FL/FW, WL2/ HW, GW/EW, LOD/MOD, HBW/HTW, and EL/MOD) were noted.

In the study, the smallest worker stingless bees were recorded in Aroroy, Masbate (unidentified species of *Tetragonula*) (3.496 + 0.17 mm), while the biggest was found in Pangi, Gasan, Marinduque (*T. iridipennis*) (4.62 + 0.32 mm). However, these variations in body length were significant between and among stingless bee populations. The worker body size has been generally considered as an adaptation to foraging activity and floral resource exploitation. Larger species, on the other hand, have a greater capacity to migrate between and among environmental fragments, while the smaller stingless bees depend only on the available resources within their jurisdiction.

General Characteristics. The workers of each bee species found in the study showed varied morphological characteristics. *T. laeviceps* have a 3.89 - 4.30 mm long body, of which the smallest bees were collected in

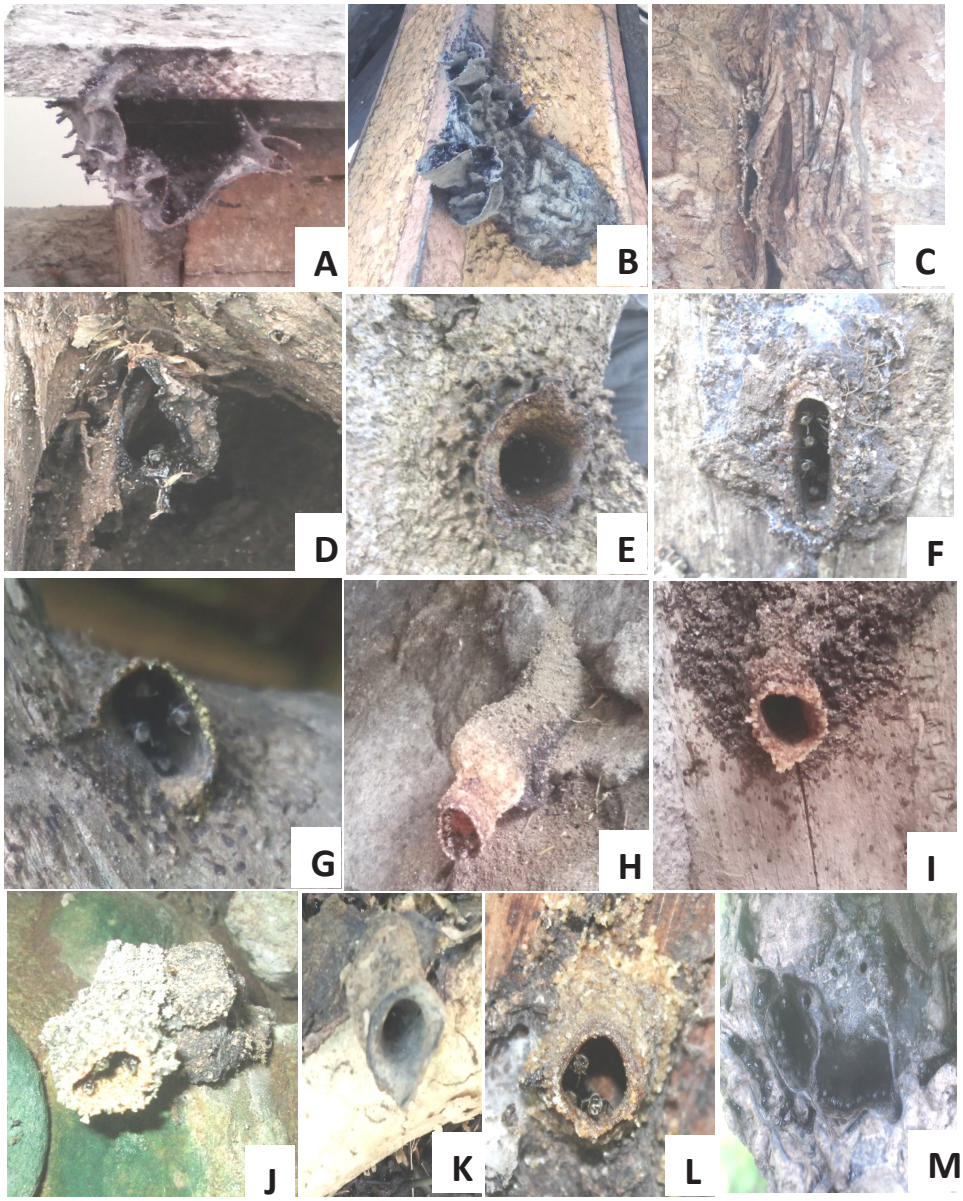

Fig. 1. Nest entrances of *Tetragonula* in Luzon Islands: A. Alabat, Quezon; B. Perez, Quezon; C. Viga, Catanduanes; D & E. Garchitorena, Cam. Sur; F& G. Aroroy & P lacer, Masbate; H & I. Gasan, Marinduque; J & K.Caramoan, Cam.Sur and L&M. Calapan and Naujan,

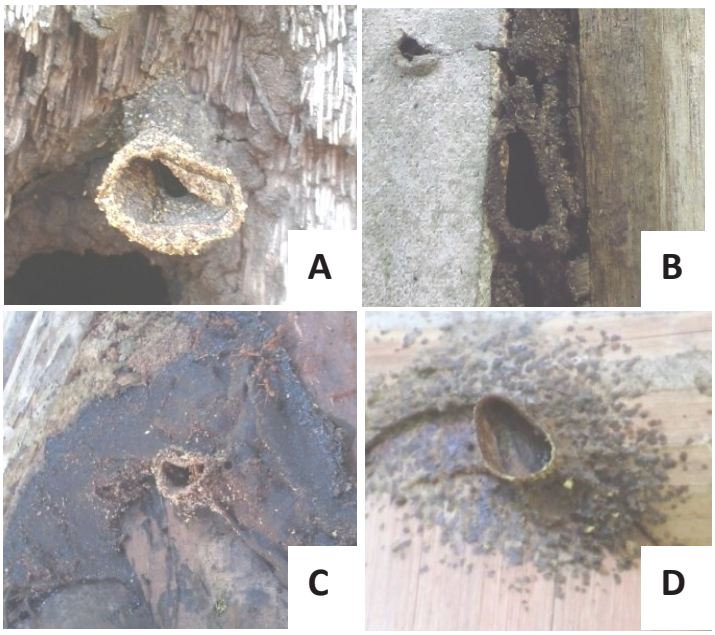

Fig. 2. Nest entrances of *Tetragonula* found in Visayas Islands (A. Hernani , Samar, B. Tagbilaran, Bohol; C. Marbuena Island, Ajuy, Iloilo; and D. Nasidman, Iloilo).

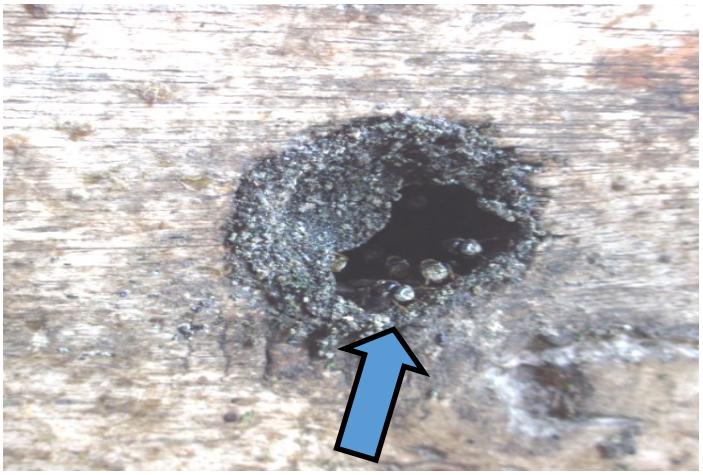

Fig. 3. Nest entrances of *Tetragonula* found in Tagum, Davao (Mindanao).

Fig. 4. Adult workers of *Tetragonula* species.
A. *T. laeviceps*; B. *T. sapiens*; C. *T. iridipennis*;
D. *Tetragonula* sp. (Unidentified species).

Caramoan, Camarines Sur while the biggest was found in Marbuena Island, Ajuy, Iloilo. Their body is characterized by black color, mesoscutum is hairy, the vertex is blackish and not banded, and anterior hind tibia is hairy (Figure 4A).

Workers of *T. sapiens* have 3.8 - 6.0 mm long body, black colored body, brown metasoma, first and the second tergum blackish-brown and blackish in the apical black mesoscutum with dark to blackish-brown hairs (Figure 4B).

Workers of *T. iridipennis* have 3.50 - 4.60 mm long body with black clored body. Mesoscutum is hairy, vertex blackish with 4-6 banded and anterior hind tibia with dark to blackish-brown hairs, while the posterior is brownish-yellow (Figure 4C).

Workers of unidentified species of *Tetragonula* have 3.3— 3.80 mm long body, the smallest among the species collected in different islands of the Philippines. Characterized by an enlarged compound eye, black-brown body color, brown metasoma, first and the second tergum blackish -brown, and blackish in the apical, slightly banded. The anterior hind tibia is characterized by blackish-brown hairs, while the posterior is blackish-yellow (Figure 4D).

Head and its Appendages. From the results of the stepwise analysis, the majority of the variables that best discriminate the group samples were found in the head (11 characters). These are the maximum width of head, lower inter-orbital distance, scape length, the maximum width of gena, length and width of flagellomeres IV, width and length of compound eyes, length of the malar area, maximum interorbital distance, interocellar andocellocular distance. It was seen

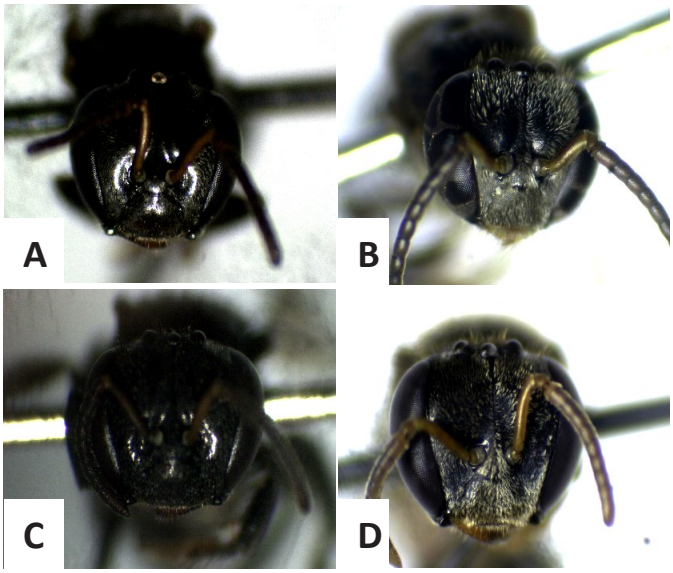

Fig. 5. Head structure of *Tetragonula* species. A. *T. laeviceps;* B. *T. sapiens;* C. *T. iridipennis;* and D. *Tetragonula sp.* (Unidentified species).

that workers of *T. iridipennis* as well as and *T. laeviceps* have overlapping characters. The width of their head, length of the malar area, lower inter-orbital distance, interocellar and ocellocular distance, and the length and width of flagellomeres IV are identical with 1.74, 0.25, 1.18, 0.26, 0.53, 0.13, and 0.13 mm, respectively (Figure 5).

However, the maximum width of gena (0.25 mm) and interocellar distance (0.26 mm) are the only characters that showed similarity between *T.laeviceps* and *T. sapiens* . This means that the above mentioned characters were not useful in identifying the species of *T. iridipennis, T. laeviceps* and *T. sapiens.* On the other hand, it was noted that the length and width of their compound eye, the maximum interorbital distance, and the length of scape became an independent variables among and between the three species collected (Figure 6).

This indicates that *T. iridipennis, T. laeviceps* and *T. sapiens* could be easily separated using these morphological characters.

Thorax and its Appendages. Among the 27 characters identified by the stepwise analysis as significant variables in discriminating the stingless bee populations in the Philippines, four have been recorded in the thorax .

These are the length and width of hind tibia, maximum width of hind basitarsus and the distance between *M-Cu* bifurcation and basal tip of marginal cell of forewing (Figure 7).

However, it was recorded that only the width of hind tibia and basitarsus were identified as distinguishable characters among the three species, *T. iridipennis, T. laeviceps* and *T. sapiens,* since the length of their hind tibia were recorded similar in *T. laeviceps* and

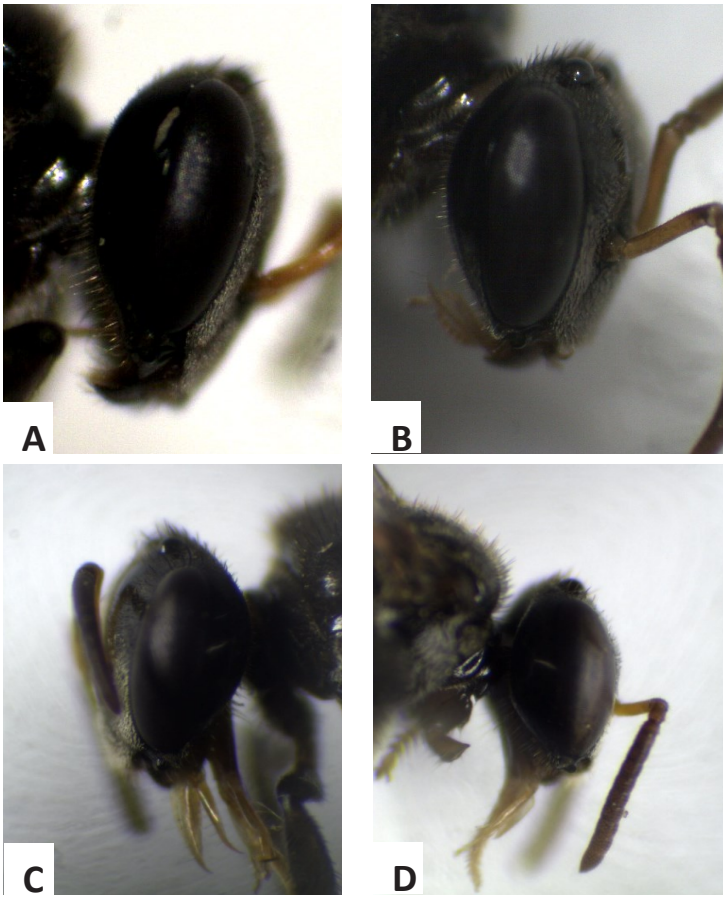

Fig. 6. Compound eye of *Tetragonula* species A. *T. laeviceps*; B. *T. sapiens*; B. *T. iridipennis*; and D. *Tetragonula* sp. (Unidentified species).

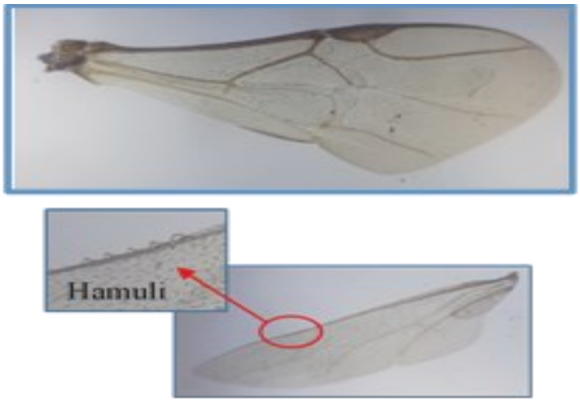

Fig. 7. Forewings (A) and Hindwings (B) of stingless bee populations in the Philippines.

T. sapiens (1.64 mm). Likewise, the distance between *M-Cu* bifurcation and basal tip of marginal cell of forewing were recorded identical in *T. iridipennis* and *T. sapiens* (1.14 mm).

Similarly, it was recorded that all examined specimens showed a clear, monotone and transparent types of membranous wings (Figure 7A). Likewise, it was found out that all collected bees had five (5) hamuli (Figure 7B). These characteristics were first recognized as distinct features for *Tetragonula* species.

Canonical Discriminant Analysis

There are four distinct clusters revealed for the entire population of the stingless bees from the scatter plot for the 1st and 2nd canonical discriminant functions analysis (CDA), based on the reduced number of characters resulted from the stepwise analysis (Figure 8). The two functions, however, were correctly classified 83.3 percent of the original groupings.

The first cluster which shares their common characters were recorded in groups 13, 14, and 18, which were collected in Ogbong, Viga, Catanduanes and Pangi, Gasan, Marinduque (1&2). These group of bees, however, share their common morphological characters in terms of the size of their head (width) (1.9 mm), compound eye (width) (0.5 mm), length of scape (seen laterally) (0.5 mm), lower interorbital distance (1.2 mm) and the distance between M -Cu bifurcation and the basal tip of the marginal cell (1.2 mm).

Unlike the latter cluster, groups 8, 9, 10, 11, and 12 were more closely related to each other. This cluster showed the overlapping of the group of *T. laeviceps* and *T. sapiens* which were found from the islands of Iloilo (Marbuena and Nasidman), Samar (Hernani), Davao (Tagum), and Bohol (Tagbilaran). These groups, however, share their common characteristics in terms of the size of their compound eye (EL) (1.2 mm), interocellar distance (IOD) (0.3 mm), ocellocular distance (OOD) (0.5 mm), length of malar area (ML) (0.3 mm) and the distance between *M-Cu* bifurcation and the basal tip of the marginal cell of the forewing (WL2) (1.1mm). Likewise, similar measurements were recorded in relation to the following: (1) wing length to head width (WL2/ HW) (0.6 mm), (2) lower interorbital distance to maximum interorbital distance (LOD/MOD) (1.1 mm), (3) interocellar distance to ocellocular distance (IOD/OOD) (0.5 mm), (4) malar length to flagellomere (IV) width (ML/FW) (2 mm).

However, eight samples 1, 2, 3, 4, 6, 7, 15, 16, and 17) occupied the third cluster, which slightly overlapped with each other and nearest to the centroid. Colonies from groups 1 to 7 were found in the islands of Camarines Sur (Caramoan and Garchitorena) and Mindoro (Calapan and Naujan) of which two species *T. iridipennis* and *T. laeviceps* were identified while the remaining groups were found in the islands of Masbate (Placer) and Quezon (Alabat and Perez) which are recorded belonging to *T. iridipennis* populations.

Furthermore, group 5 was distinctly separated from the other groups. Sample specimens from

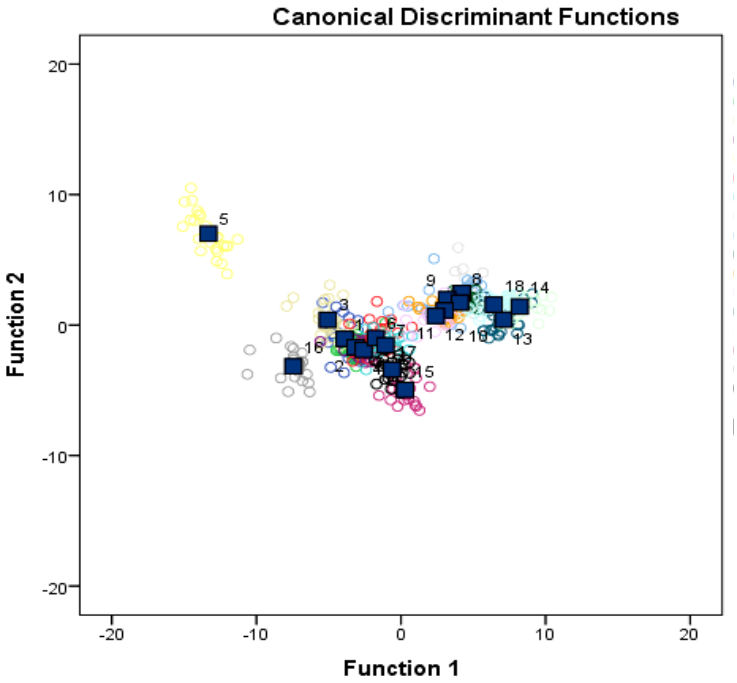

Fig. 8. Scatterplot of the Canonical Discriminant Analysis (CDA) showing the clustering of *Tetragonula* sp.

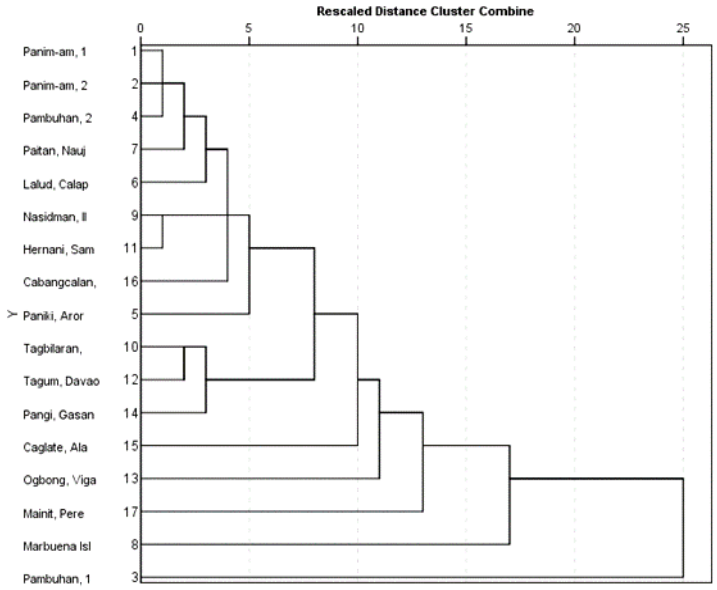

Fig. 9. Neighbor-joining dendogram generated from the Euclidean distances between the centroids of CDA indicating probable distribution of *Tetragonula* populations in the Philippines.

group were collected from the island of Masbate (Aroroy). This group was recorded as the farthest among all distances between any groups. These bees, however, were morphologically identified as belonging to the genus *Tetragonula*. No similar record, however, matches their unique characteristics which means that a possible new species of *Tetragonula* was found on the island of Masbate.

The graphical representation of CDA scores shows a number of subpopulations within the species in the different collection sites. Ecotype distributions seem to be pulling along latitudinal gradient under the influence of a number of environmental variables.

Species Group Relationships and Similarities

The results of the neighbor-joining dendrogram generated from the Euclidean distances between the centroids of CDA highlighted the species relationship and similarities of various sample populations of stingless bees collected in the different islands of the Philippines. The graphical representation show clear proximities of various populations with each other (Figure 9). Among the group populations, sample specimens from Camarines Sur (Caramoan 1 & 2 and Garchitorena 1) and Mindoro (Naujan and Calapan) were recorded as the closest group. Thus, specimens from this group were identified to belong to *T. iridipennis* populations.

The dendrogram separated the adult workers of stingless bees from Tagbilaran, Bohol & Tagum, Davao. This group was confirmed as belonging to *T. sapiens*. Similar observations were noted in samples from Nasidman, Iloilo, and Hernani, Samar which are identified as belonging to *T. laeviceps*. Distances from these groups were, however, found in a range of 0 to 5.

The results indicated that the morphological characters are useful in the separation of the two species *T. laeviceps* and *T. sapiens.* But for *T. iridipennis* group, the results of the dendrogram showed that their populations were not separated due to several morphological variations. Therefore, the separation of species should use the nest structure, and DNA sequencing besides morphological characters.

Species Identified using Gene Sequencing

Out of 18 stingless bees samples, only 12 samples were submitted for DNA sequencing analysis due to insufficient numbers. Samples from groups 13 to 18 were collected in Ogbong, Viga, Catanduanes, Pangi, Gasan, Marinduque, Caglate, Alabat, Quezon, and Mainit, Perez, Quezon were not included in DNA sequence analysis.

Basic Local Alignment Search Tool (BLAST) analysis revealed that only amplicon from 16S RNA genes was reproducible in 12 colonies analyzed. However, no hits were found in samples from Panim-an, Caramoan, Camarines Sur and Oriental, Mindoro (Calapan and Naujan). This is an indication of a new possible strand or subspecies as the output of the study.

The BLAST searches showed that forward sequence from Tagbilaran, Bohol has 97 percent identify with the 16S rRNA genes found in the Old World Stingless bee, *Tetragonula sapiens.* Likewise, an almost similar percentage was recorded in samples from Tagum, Davao with 97.3 percent (Forward) and 92.1 percent (Reverse) identity with 16S rRNA genes found in the same species. This means that samples from these islands are confirmed to belong to *T. sapiens.*

However, the two-sample specimens from Panbuhan, Garchitorena, Camarines Sur (1&2), showed both forward (92.3%) and reverse (90.2 & 92.6%) sequence similarity with the 16S rRNA genes found in *Tetragonula pagdeni* while the samples from Paniki, Aroroy, Masbate were found 94.5 and 94.1 percent (Forward and Reverse) in the old world stingless bees, *Tetragonula clypearis*. However, the rest of the samples collected in the different islands of the Philippines shared their genetic similarity in *T. sapiens*.

No studies on the gene sequencing of stingless bees currently exists. Moreover, it is important that the information generated must be submitted to the database for the BLAST Analysis of stingless bees.

Species Confirmation

Although the nest of stingless bees is often used as a key feature to solve taxonomic problems (Dollin et al*.,* 1997), the results of the study suggest that nesting habits of stingless bees and the structures of their entrances were not species-specific due to close similarities and habits.

Likewise, due to the absence of reliable structural characters in the workers (Sakagami, 1978), sorting of stingless bees species collected became the main problem in the study. However, Sakagami (1978) suggested that the classification must depend on the size, coloration, proportion, and pilosity, which the study followed and used together with the results of BLAST analysis.

Thus, the results of the study revealed that there were three (3) species and an unidentified species of stingless bees where the samples were classified or identified. All species noted belong to the genus *Tetragonula*. The genus was characterized by the small body size and could be found throughout the world with 500 species (Ramirez et al.,2010). The genus *Tetragonula* were likewise recorded in the continental Asia, Sri Lanka, India, Southeast Asia , Thailand, Malaysia (Sakagami, 1978; Rasmussen and Michener 2010).

Three species of stingless bees were found in the provinces of Camarines Sur and Mindoro (*T. laeviceps* and *T. iridipennis)* while the rest of the species were distributed throughout the country.

In the study, the highest number of colonies found was *T. iridipennis* (9 colonies), followed by *T. laeviceps* and *T. sapiens* with six and two colonies, respectively.

In particular, each species showed specific nest requirements

based on their habitat quality. The *T. sapiens* colonies were exclusively found in the parts of the houses, such as wall and drawer while colonies of *T. laeviceps* and *T. irridipennis* build their nest in hard, solid, unbreakable, and permanent objects like in the crevices of the living tree trunk, decaying logs, cemented wall, and rocks.

The highest number of *T. iridipennis* colonies were found in crevices of tree trunks (Miyapi and Narra) (3 colonies) followed by decaying logs (2 colonies), stone cavity, wooden wall, cemented post, and fern. However, no specific nesting sites were recorded in *T. laeviceps*.

The characteristics of nest entrances of *T. laeviceps* are tubular and round-ringed. The colors are light brown, brown, and black with both soft and hard rigidity. Nest entrances of *T. iridipennis* are characterized by irregular shapes. Others are tubular, round-ringed, light to dark brown in color, and soft and hard rigidity. Circular shape, black in color, and soft rigidity are characters of nest entrance of *T. sapiens,* while, dark brown in color, ovular in shape, and soft rigidity are characters observed in the nest entrance of an unidentified species. However, all entrance holes were observed facing east with 3 to 15 guards. However, considering the BLAST results, it could be inferred that only samples from Tagbilaran (Bohol) and Tagum (Davao) were confirmed similar to *T. sapiens*. However, samples from Panbuhan (Garchitorena), Paniki (Aroroy, Masbate), Marbuena and Nasidman (Iloilo) and Hernani (Samar) were not similar to *T. pagdeni,T. clypearis)* and *T. sapiens,* respectively, since the values are less than 97 percent.

The results indicated that sample specimens from these groups are confirmed to be *Tetragonula* Moure considering the available data base for the stingless bees.

On the other hand, the results of the morphometric analysis revealed that five specimens collected in Caramoan, Camarines Sur, Paitan, Naujan, Oriental Mindoro, Hernani, Samar, Marbuena, and Nasidmen Iloilo, had great similarity on workers of *T. laeviceps*. Likewise, nine populations were similar to *T.iridipennis* and two groups similar to *T. sapiens* and one unidentified species coded as *Tetragonula* sp.

Using the QGIS Version 2.16.1 program, different locations where stingless bee samples were collected have been plotted and a distribution map was generated. The distribution map showed the areas where specific species of stingless bees could be found (Figure 10).

Presence of *T. sapiens* was noted in Tagbilaran, Bohol, and Tagum, Davao which were collected in the crevices of the wall and file cabinet inside the house. However, *T. iridipennis* found in the island of Masbate (Placer), Camarines Sur (Garchitorena), Oriental Mindoro (Calapan), Catanduanes (Viga), Marinduque (Gasan), and Quezon (Alabat and Perez), which are collected in cavities of Miyapi tree, decaying wood, narra, cemented wall and rocks.

T. laeviceps, however, was recorded in Caramoan (Camarines Sur) Paitan, Naujan (Oriental Mindoro) Hernani (Samar,) Marbuena, and Nasidman (Iloilo),

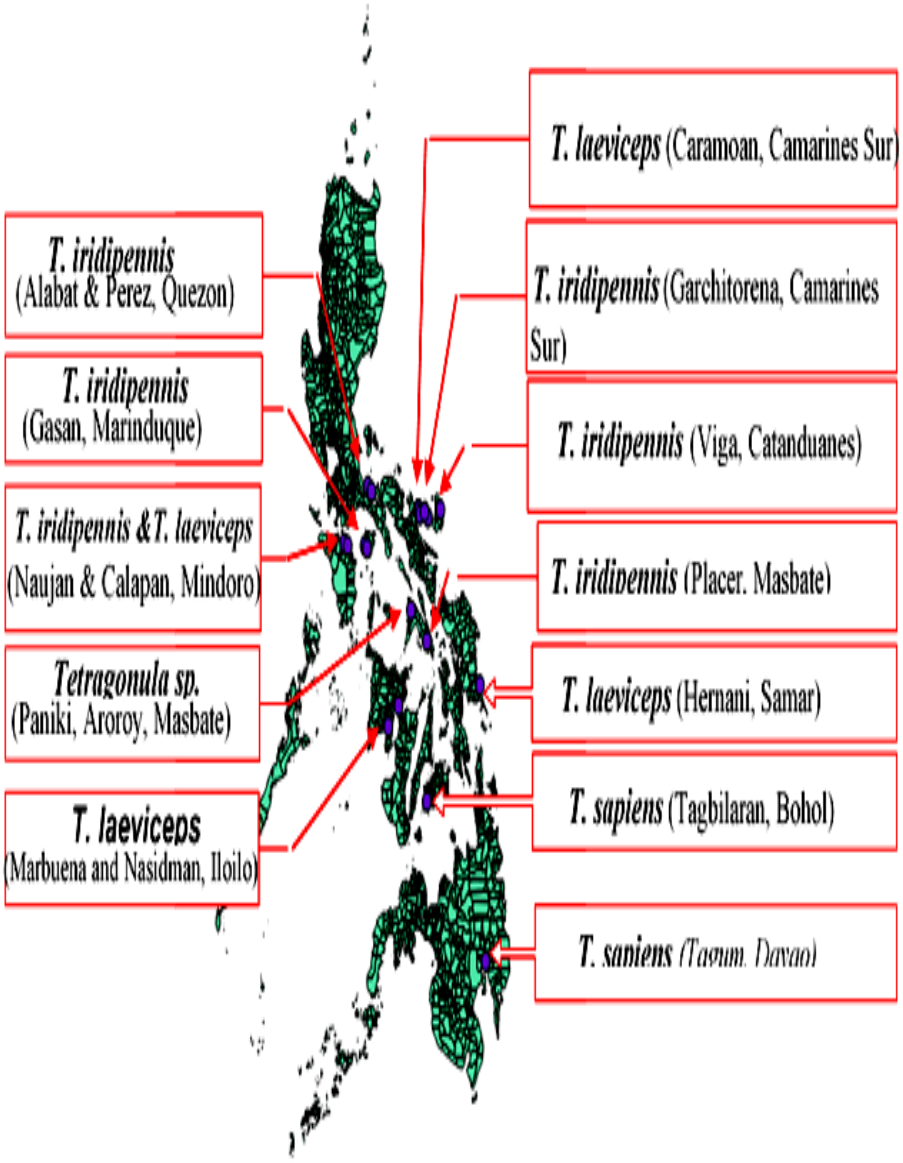

Fig. 10. Philippine map showing the collection sites with the identified stingless bees species.

while in Aroroy (Masbate) , an unidentified species was observed.

The distribution map would be useful in understanding the existence of stingless bees in a certain area and the possible effects of geographical differences on their morphological features.

CONCLUSIONS

The morphometric characters were used as an effective tool for discriminating *Tetragonula* species.

Samples from Masbate (Aroroy & Cabangcalan) were distinctly separated from the other groups.

The only amplicon from 16S rRNA genes was reproducible in 12 colonies analyzed. No hits were found in samples specimen from Panim-an, Caramoan, Camarines Sur (1&2), and Oriental, Mindoro (Calapan and Naujan), however, samples from Tagbilaran, Bohol, and Tagum, Davao were confirmed.

In Luzon, the identified species of stingless bees in the islands was *T. iridipennis* found in Alabat and Perez (Quezon), Gasan (Marinduque), Garchitorena (Camarines Sur), Viga (Catanduanes), Placer (Masbate), and Naujan (Mindoro) while *T. laeviceps* were found in Calapan (Mindoro), Caramoan (Camarines Sur). For Visayas, the identified species were *T. laeviceps* in Hernani (Samar), Marbuena, and Nasidman (Iloilo) while *T. sapiens* in Tagbilaran (Bohol). In Mindanao, the species was identified as *T. sapiens* in Tagum (Davao). An unidentified species was found in Paniki (Aroroy, Masbate).

Stingless bees preferred cavities to build their colonies in more secured, hard, solid, unbreakable, and permanent objects, nesting habits of the stingless bees collected were not species-specific.

To solve problems regarding stingless bees similarities and relationships, the following recommendations are: 1) There is a need to study on adaptations and distribution of the stingless bees considering its morphology, nesting habits, and nest architecture; 2) The results of the molecular analysis of the different stingless bees species found in the study must be inputted in the database for Indo-Malayan bees, however, further sequencing of the whole genome using mitochondrial analysis must be done to complete the data on the stingless bees particularly in the Philippines; *3)* It is also recommended that another similar study be conducted using similar methodology focusing the whole Islands of the country.

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